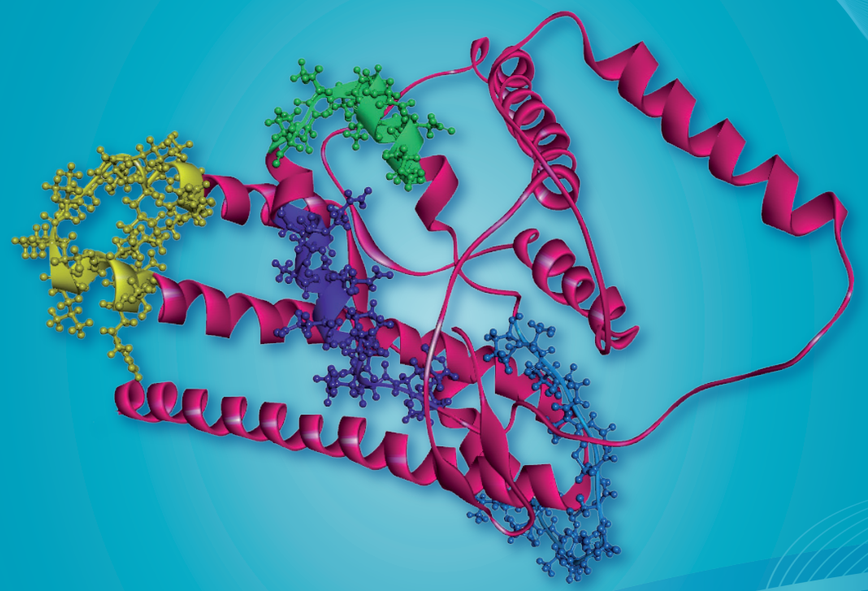
VECTOR CONTROL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)



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2014
Annual Report

PUDUCHERRY

WHO Collaborating Centre for Research and Training
in Lymphatic Filariasis and Integrated Methods
of Vector Control

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(INDIAN COUNCIL OF MEDICAL RESEARCH)

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PREFACE

The Centre continued to focus on research on elimination of lymphatic filariasis (ELF) and development of site specific control strategies for the control of malaria, dengue and JE. Most of the projects were carried out in close collaboration with State Health Departments and other ICMR and non-ICMR Institutes. During the year, a total of seven projects were completed, 18 ongoing and 14 newly proposed. More than 80% were on operational research and others include translational and basic research. The Centre has maintained its publication profile with 37 research papers including 5 in press/accepted in peer reviewed National and International Journals.

The Centre has taken a lead role, with the support of other ICMR Institutes, in carrying out independent appraisal of the progress of National Programme on ELF. The xenomonitoring protocol as an alternative/supplement to Transmission Assessment Survey for post-MDA surveillance is being validated. In a collaborative venture with VIT, Vellore, efforts are under way to develop a portable miniature electrochemical detector of *W. bancrofti* infection in vectors.

Studies on the therapeutic efficacy of Artesunate Combination Therapy (ACT) and comparative efficacy of 50% DDT and 75% DDT WDP formulations have been completed. The outcome of these studies contribute to the drug/insecticide policy of the National Vector Borne Disease Control Programme. Studies among the Kani forest tribes of Thiruvananthapuram district, Kerala, indicate indigenous transmission of *Leishmania donovani* with cutaneous manifestations. Natural infections of *L. donovani* were detected in wild caught *P. argentipes* females, and in domestic dogs. Using remote sensing & GIS, a method has been developed to forecast the risk of JE transmission in terms of mosquito vector abundance in paddy grown areas.

Under the tribal health research initiatives of ICMR, "Tribal Health Research Unit" has been sanctioned for the Centre. In view of increasing trend of re-emerging vector borne diseases, expertise and facilities for laboratory diagnosis of scrub typhus and vector surveillance have been established. A preliminary study on KFD vectors has been initiated in collaboration with NIV, Pune. Under the translational research, a secondary metabolite of an indigenous isolate of *Bacillus amyloliquefaciens* with mosquito larvicidal and pupicidal activity was evaluated for its antibacterial effect on clinical isolate of *Staphylococcus aureus.* Complete specification for improved process developed at VCRC for production of Cyclosporine A from the fungus, *Tolypocladium sp.* (VCRC F21) has been filed. Biomedical Informatics Centre (BMIC) started functioning at VCRC since January 2014 and was engaged in molecular modelling studies with strong linkage with the ongoing and newly developed laboratory studies on product development. Construction of a VectorInfo database is in progress.

During the year, fourth batch of students were admitted to M.Sc. Public Health Entomology course (affiliated to Pondicherry University), and one candidate from Maldives was sponsored by WHO. In view of the short of hands, the training programme had to be limited to the students and faculties of local Medical colleges.

The fund flow continued to be low during this year, too. However, care was taken to manage all the important activities, with the support of the Secretary to the Government of India, Department of Health Research and Director General of ICMR and the Administrative Division of ICMR, New Delhi. A number of scientists and staff retired from service on superannuation during this year. While steps have been initiated for new recruitment, the ongoing research / HRD programmes were continued with the extended support of some of senior scientists and administrative staff on consultancy mode. The Centre received Rs. 170.8 lakhs from ICMR, out of which about Rs. 96 lakhs were spent on intramural research activities. A sum of Rs. 546 lakhs was generated from various extramural projects. The centre received support from ICMR Task Force, VBDSF & THRF, National Vector-Borne Disease Control Programme (NVBDCP), New Delhi, Department of Science and Technology (Govt. of India), WHO, Geneva, WHO-SEARO and LF Support Centre-Bill & Melinda Gates Foundation for various research programmes.

The Scientific Advisory Committee (SAC) has been reconstituted and the remarkable contribution by the outgoing SAC Members in guiding the research activities is gratefully acknowledged. We wish to thank members of various institutional committees of the Centre who have supported the Institution Building activities. We also thank the Directorate of National Vector-Borne Disease Control Programme (NVBDCP), New Delhi, Dept. of Health & Family Welfare, Govt. of Puducherry, Tamil Nadu, Kerala and Odisha for their continued support to the field research activities of the Centre.

I am very much thankful to all the scientists and staff of our Centre for their tremendous support and contribution for the successful accomplishments of the Centre.

Dr. P. Jambulingam
Director

प्रस्तावना

केंद्र द्वारा राष्ट्रीय कार्यक्रम की सुबिधा के लिए लसीका फाइलेरिया (LF) के उन्मूलन पर अनुसंधान गतिबिधिओं पर ध्यान केन्द्रित जारी रखा और राज्यों के स्वास्थ्य बिभाग और अन्य राष्ट्रीय संस्थानों के सहयोग से मेलेरिया, ईंगू और जेई (JE) के रोक थाम के लिए क्षेत्र बिशिष्ट रणनीतियाँ बिकसित किया । अधिकांश परियोजनाओं राज्य के स्वास्थ्य विभागों और भारतीय आयुर्विद्यान अनुसंधान परिषद और गैर-भारतीय आयुर्विद्यान अनुसंधान परिषद केन्द्रों के निकट सहयोग के साथ से किया गया। ईस वर्ष के दौरान कुल 7 परियोजनाओं सम्पूर्ण हुआ, 18 चल रही हे और 17 नये परियोजनयों का कार्य शुरू कि गई है, जिसमें प्रायोगिक (80%), ट्रांसलेसलनल और मौलीक अनुसंधान सामील है। अनुसंधान के निष्कर्षों राष्ट्रीय और अंतरराष्ट्रीय पत्रिकाओं द्वारा समीक्षा के बाद कूल 37 प्रकाशित हुआ जिसमें अभी 5 प्रैस में है ।

केंद्र द्वारा देश में LF कार्यक्रम की स्वतंत्र मूल्यांकन में भारतीय आयुर्विज्ञान अनुसंधान परिषद के अन्य संस्थानों के समर्थन के साथ एक प्रमुख भूमिका लिया है । पोस्ट- MDA निगरानी के लिए संचरण आकरन सर्बेक्षण के एक विकल्प में Xenomonitoring प्रोटोकाल की उपयोगिता मान्य किया जा रहा है । VIT, भेलोर के साथ एक सहयोगी उपक्रम में केंद्र वेक्टर में *W.bancrofti* संचरण का एक लघु ईलेक्ट्रो केमिकल डिटेक्टर को बिकसित करने में सफल रहा हें।

आर्टिसुनेट संयोजन चिकित्सा (ACT) कि चिकित्सीय प्रभावकारिता, 50% और 75% DDT WDP योगों का तुलनात्मक प्रभावकारिता की पर अध्ययनों किया गया हैं। इन अध्ययनों के परिणाम राष्ट्रीय वेक्टर जनित रोग नियंत्रण कार्यक्रम के दवा/कीटनाशक नीति के लिए उपयोगी होगा । तिरुवनंतपुरम जिले, केरेल, की कानी बन जनजातियों के बीच एक अध्ययन शुरू किया गया है, जिसमे *Leishmania donovani* के त्वचीय अभिव्यक्ति के स्वदेशी संचरण का संकेत पाया गया हें। *Leishmania donovani* के प्राकृतिक संचरण जंगलीप्राप्त फ़ीमेल *P.argentipes* और पालतू कुत्तों मे पाया गया है । सुदूर संबेदन और भौगलिक सूचना प्रणाली (GIS) उपयोग करके धान लगाया क्षेत्रों में मच्छर वेक्टर बहुतयात और JE संचरण के जोखिम का पुर्बानुमान करने के लिए एक बिधि विकसित किया गया है ।

ICMR जनजातीय स्वास्थ्य अनुसंधान के तहत 'आदिवासी स्वास्थ्य अनुसंधान इकाई' ईस केंद्र के लिए मंजूर किया गया है। फिर से उभरते हुए वेक्टर जनित रोगों के ध्यान में रखते हुए, Scrup typus निदान और बेक्टर निगरानी के लिए विशेषज्ञता और निदान के लिए प्रयोगशाला के सुबीधायों की स्थापना की गई है । KFD वेक्टर पर एक प्रारम्भिक अध्ययन, NIV, पुणे के सहयोग से शुरू किया गया है । ट्रांसलेसनाल शोध के तहत, स्वदेशी *Bacillus amyloliquefaciens* से प्राप्त सेकंडरी मेटाबोलाइट मच्छर larvicidal और pupicidal गतिविधि मानब रोगजनक *Staphylococcus aureus* के खिलाफ उपयोगिता पाया गया है । Fungus *Tolypocladium sp* (VCRC F21) से Cyclosporine A उत्पादन के लिए VCRC द्वारा पूर्ण बिबरण विकसित सुधार प्रकिया पैटंट दायर की गई है । जैव आयुर्विज्ञान सूचना केंद्र (BMIC) जनवरी 2014 से VCRC में शुरू हुआ और यह मलिकुलर मॉडलिंग अध्ययनों, नव विकसित प्रयोगशाला अध्ययन और उत्पाद विकास के साथ मजबूत संबंध पर कार्य जारी है । VectorInfo डाटा बेस के निर्माण कार्य जारी है ।

ईस वर्ष के दौरान छात्रों के चौथे बैच एम. एससी (लोक स्वास्थ्य किट विज्ञान, पण्डिच्चेरी विश्वविद्यालय से संबद्ध पाठ्यक्रम) और मालदीप से एक ऊमीदबर WHO द्वारा प्रायोजित किया गया था । कर्मचारीओं की कमी को देखते हुए, प्रशिक्षण कार्यक्रम में छात्रों और स्थानीय मेडिकल कालेज के लिए सीमित किया गया है ।

ईस बर्ष भी फंड की कमी जारी रहा। लेकिन, सचिब, स्वास्थ्य अनुसंधान विभाग, भारत सरकार, और भारतीय अनुसंधान परिषद, नई दिल्ली के महानिदेशक और प्रशासनिक विभाग के समर्थन के साथ सभी महत्यपूर्ण गतिविधियों जारी रहा। वहु संख्या में वैज्ञानिक और कर्मचारियों इस वर्ष के दौरान सेवानिवृत्ति हुए। जबकि नई कर्मचारीयों के भर्ती लिए कार्य शुरू की गई है, चल रहे अनुसंधान / मानव संसाधन विकास कार्यक्रम परामर्श मोड पर कुछ वरिष्ठ वैज्ञानिक और प्रशासन कर्मचारीयों की समर्थन के साथ जारी रखा गया । ईस साल के दौरान केंद्र को ICMR से 170.8 लाख रुपए मिला जिसमें से करीब 96 लाख रुपए अंदर का अनुसंधान गतिविधियों पर खर्च किए गए थे। रुपए 546 लाख की राशि विभिन्न बाह्य परियोजनाओं से प्राप्त हुआ । केंद्र ICMR टास्क फोर्स, VBDSF और THRF, राष्ट्रीय वेक्टर जनित रोग नियंत्रण कार्यक्रम (NVBDCP, नई दिल्ली), विज्ञान और प्रौद्योगिकी विभाग (भारत सरकार), WHO, जेनेवा, WHO-SEARO और बिल एवं मेलिंडा गेट्स फाउंडेशन LF समर्थन केंद्र द्वारा विभिन्न अनुसंधान कार्यक्रमों को समर्थन मिला।

वैज्ञानिक सलाहकार समिति (SAC) का पुनर्गठन किया गया है और अनुसंधान गतिविधियों का मार्गदर्शन में निवर्तमान SAC सदस्य द्वारा उल्लेखनीय योगदान के लिए कृतज्ञता ज्ञापन करते हें। हम विभिन्न संस्थागत समितियों की सदस्यों को केंद्र की विभिन्न गतिविधियों का समर्थन पर धन्यवाद करना चाहते हैं । हम राष्ट्रीय वेक्टर जनित रोग नियंत्रण कार्यक्रम निदेशालय (NVBDCP, नई दिल्ली), स्वास्थ्य और परिवार कल्याण विभाग, पांडिचेरी, तमिलनाडु, केरल और उड़ीसा सरकार, केंद्र के क्षत्रिय अनुसंधान गतिविधियों के लिए उनके निरंतर समर्थन पर धन्यवाद करना चाहते हैं ।

में, केंद्र के सफल उपलब्धियों के लिए जबरद्स्त समर्थन और योगदान पर केंद्र के सभी वैज्ञानिक और कर्मचारियों पर बहूत बहूत आभारी हूँ ।

डॉ.पी.जंबुलिंगम
निदेशक

EXECUTIVE SUMMARY

The research activities of the Centre continued to focus on the development of tools and strategies to support the national agenda towards the elimination of lymphatic filariasis, besides malaria, dengue and JE. Efforts have also been taken to initiate vector surveillance and identification of areas potential for risk of the re-emerging vector borne diseases like Scrub typhus & Kyasanur Forest Disease. The major activities carried out at the Centre are summarised below.

Lymphatic Filariasis

- The process of miniaturization of the electrochemical detector of *W. bancrofti* infection in vectors, developed at VCRC in collaboration with VIT Vellore is in progress. The electrochemical impedence spectroscopy (EIS) signal measurement was found to have reproducibility at 1 pM of the probe concentration and at 10 pM of the target ss-DNA of the parasite. The approach will lead to a portable miniature detector.
- Sampling strategies for xenomonitoring of infection in *Culex* vector by PCR as a surveillance tool for assessing post-MDA situation of lymphatic filariasis elimination programme has been developed and validated. The two-stage cluster sampling (30 clusters x 10 pools) protocol involving villages and households can be used for the collection of gravid females by using traps for monitoring vector infection and infectivity. The usefulness of xenomonitoring as an alternative to TAS during post-MDA surveillance is being assessed.
- Preliminary assessment of the impact of the ongoing morbidity management and disability prevention programme for filarial lymphedema among 260 patients in Villupuram District, Tamil Nadu, showed that the programme has yielded the desired results in prevention of disability in the form of reversal of lymphedema to normal condition or remaining in the same grade without further progression (46% of grade 1, 52% of grade 2 and 92% of grade 3).
- A collaborative project with RMRC, Port Blair has been initiated on effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to mass drug administration towards elimination of the lone foci of diurnally sub-periodic *W. bancrofti* in Andaman & Nicobar Islands. Baseline data collection on mf prevalence, antigenemia prevalence among children and salt usage pattern of the community has been completed.

Malaria/Leishmaniasis

- Studies on tolerability, efficacy and operational feasibility of Artesunate combination therapy (ACT) in a tribal area of Odisha suggest that the ACT is well tolerated. The clinical and parasitological response (ACPR) was 90.7%, late parasitological failure (LPF) 8% and early treatment failure (ETF) 1.7%.The major adverse effect was abdominal pain which lasted up to three days after starting the treatment. However, this symptom was mild as it was easily tolerated by the study patients. There is a need for training of ASHA's at regular intervals and regular replenishment of RDT kits and drugs for early treatment of malaria.
- Comparative efficacy of indoor residual spraying of DDT WDP 75% and DDT WDP 50% was studied against the malaria vector, *An. Fluviatilis* in Odisha. There was no significant difference between the impact of the two formulations on indoor/outdoor daytime resting densities, light trap densities, human blood index, survival rates and vector infection rates. There was also no difference between the two formulations in their ease of application. However, the residual efficacy of sprayed surfaces and post-spray mud-plastered surfaces was higher with DDT 75% arm than with DDT 50%. The quantity of DDT 75% required for a given population is about 30% lesser than that of DDT 50%, which would considerably reduce the cost of transportation, storage space, handling etc.
- Indigenous transmission of *Leishmania donovani*, with symptoms of cutaneous leishmaniasis (CL), among the Kani forest tribes of Thiruvananthapuram district, Kerala, was established. Natural infection of *L. donovani* was detected in wild caught *P. argentipes* females through PCR assay. Blood samples from domestic dogs were positive for *L. donovani* DNA indicating their possible role as reservoir host of the parasite. Awareness on the public health importance of CL infection and prevention was created among the Kani tribes through health education.

Dengue/Chikungunya/JE

- Using remote sensing & GIS, a method has been developed to forecast the risk for JE incidence in terms of predicting increase in mosquito vector abundance for areas where conventional paddy cultivation is in practice. Different approach is required for the areas where there is mixed vegetation.
- In the research cum intervention study on JE in Gorakhpur District, initiated in 2013, baseline data on vector density, blood meal index and minimum infection rate were generated. Indoor Residual Spray (IRS) with Lambda cyhalothrin 10% WP and use of Long Lasting Insecticidal Net (LLIN) are the two intervention methods planned. Materials required for these measures are procured and is in the implementation stage.
- Demonstration of vector control and prevention of dengue/chikungunya through partnership and community empowerment showed that it is feasible to motivate the community through trained school students. Interpersonal communication is more effective than mass communication. Drastic reduction in the number of discarded containers (vector breeding sources) could be achieved in the areas under school based approach in contrast to the comparison areas.

Scrub Typhus/KFD

- Expertise and facilities for laboratory diagnosis of scrub typhus and entomological research have been developed. Preliminary studies in Puducherry showed higher levels of chigger (*Leptotrombidium deliense*) indices and infection of rodents with *Orientia tsutsugamushi* in areas where human cases of scrub typhus were reported.
- In a preliminary study on KFD in the potential risk areas of adjoining states to Karnataka, 83.7% of the ticks collected were *Haemaphysalis spinigera*. Tick samples analysed at NIV, Pune, in pools by RT-PCR were positive for KFD virus indicating the risk of transmission in these areas.

Microbial/Chemical agents for vector control

- *Bacillus cereus* strains isolated from marine soil and marine fish samples were found to be active against larval stages of mosquito vectors.
- Secondary metabolite of an indigenous isolate of *Bacillus amyloliquefaciens* was found to have mosquito larvicidal and pupicidal activity. In addition, the metabolite also showed antibacterial effect on clinical isolate of *Staphylococcus aureus*, indicating its usefulness against human pathogenic bacteria.

Translational Research

- Multi-centric evaluation of stage specific RT-PCR assay for the detection of infective larvae of lymphatic filarial parasite confirmed that it has the potential for application in the monitoring of LF transmission. The technology is posted in the ICMR website for commercialization.
- The promising six numbers of naphthoquinone analogues reported earlier for *in vitro* macrofilaricidal activity were studied further for *in vitro* ADME properties in comparison with standard drugs. These compounds were stable in the acidic pH of the stomach and have good balance between solubility and permeability for optimal oral absorption and cell membrane permeation.
- The technology for the production of mosquito larvicidal formulation from *Bacillus thuringiensis* var. *israelensis* has been licensed to one more commercial firm i.e. 11th commercial firm.
- The process for the production of aqueous suspension (AS) formulation of a mosquitocidal lipopeptide of *Bacillus subtilis sp. subtilis* (VCRC B471) was designed. Preliminary field trial indicated that the formulation yielded more than 70% reduction of pupal density and 100% inhibition of adult emergence of Anophelines for upto four days. Patent granted (# 264599) for this invention on 8th Jan, 2015.
- Complete specification for improved process developed at VCRC for production of Cyclosporine A from the fungus, *Tolypocladium sp.* (VCRC F21) has been filed on 11.3.2014.

Biomedical Informatics Centre

- ***VectorInfo***, a web-repository of medically important Indian arthropods has been developed to offer information on vectors ranging from basic biology, molecular aspects to control strategies of vectors that are known to pose serious threat to humans.
- Potential antigenic determinants were predicted on cuticular collagen protein of *Wuchereria bancrofti*and synthesized. These antigenic peptides are under in-vitro evaluation for their immunogenicity.
- A lead molecule that inhibits glutathione synthetase of *Plasmodium falciparum* was identified through virtual screening from Ligand-info library and this molecule could be considered for further evaluation towards the development of anti-malarial drugs.

Facility

- Methods have been standardized for preparation of insecticide impregnated papers for conducting susceptibility tests of vector mosquitoes with DDT, malathion, deltamethrin, permethrin, alpha cypermethrin and lambdacyhalothrin. Quality of the papers were checked and found to be similar to that of WHO papers, in terms of insecticide content. The papers are ready for multi centric validation.

Human Resource Development

Academic

M.Sc. Public Health Entomology: Twelve candidates have been admitted for the year 2014–16, fourth batch of M.Sc. Public Health Entomology course affiliated to Pondicherry University. Among these, one candidate is from Maldives and is sponsored by WHO.

Ph.D. Programme: Seventeen full time (Zoology – 10; Microbiology – 5; Chemistry - 2) and two part time internal (one each from Zoology and Microbiology) candidates continue to pursue their Ph.D. programme. Two of them have completed their work and thesis submitted to Pondicherry University.

Training

Informal: Thirty seven PG students from different medical colleges of Puducherry were offered training in areas such as epidemiological surveillance tool for VBDs, control of mosquito borne diseases, implementation of rapid response and disaster management.

Observational: 388 students from different Institutes of Puducherry, Tamil Nadu and Kerala are given orientation and exposure to various ongoing programmes of the Centre.

UNIT

1

Scientific Activities

CONTENTS

1.1.1 Morbidity management and disability prevention programme (MMDP) for filarial lymphoedema: Assessment of impact and impediments

IM 1401: Jul 2014 – Jun 2016

Das LK, De Britto RLJ, Vijayalaxmi G and Krishnakumari A

There are about 800,000 filarial lymphoedema cases in India. The National Programme in India has envisaged to eliminate Lymphatic filariasis by 2015. The two strategies: Mass Drug Administration (MDA) of annual single dose with DEC and Albendazole to interrupt transmission and providing morbidity management to diseased people to prevent disability have been initiated since 2004. The morbidity management strategy for disability prevention (MMDP) for lymphoedema includes limb hygiene, prevention of entry lesions, limb elevation, simple exercise, management of acute attacks and hydrocelectomy for hydrocele cases. However, this component has not yielded the desired results due to barriers preventing the sustainability of the programme at the end user and provider levels. The assessment parameters have not been standardized for monitoring and evaluation of this programme. This study aims at addressing some of these issues with the following objectives.

Objectives:

- Assessment of the impact of ongoing MMDP programme on prevention of disability.
- Identifying impediments for sustainability of the ongoing MMDP programme.
- Identifying suitable parameters for evaluation of the impact of MMDP Programme.

Age, lymphoedema grade and duration of disease matched patients are being recruited in three cohorts. Patients in cohort-1 are recruited from VCRC field study villages (1993-2004) in Villupuram district and handed over to TN Govt. in 2004 for continuation of MMDP programme. These patients will be assessed for impact, impediments and sustainability of the programme. Patients in cohort-2 are recruited from villages in Villupuram district under MMDP by TN Govt. and will be assessed for impact and impediments. Patients in cohort-3 are recruited from villages in Villupuram district to whom VCRC will provide MMDP package and monitor for its compliance, impact, impediments and sustainability.

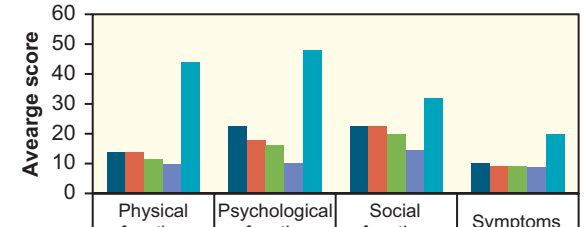
Recruitment of study patients: A total of 546 filarial lymphoedema patients were enumerated in 31 villages of Tindivanam and Gingee taluks of Villupuram district of Tamil Nadu, of which 287 cases were recruited for the study (cohort-1 with 100 cases-mean age 53.9 ± 9.1 years, cohort-2 with 104 cases-mean age 55.7 ± 7.9 years and cohort-3 with 83 cases-mean age 54.0 ± 9.7 years).

Baseline line data was collected from the patients in cohort-3 (VCRC intervention group). A total of 67 filarial lymphoedema patients were interviewed using a structured and pre tested questionnaire. Majority (95%) of these patients seek treatment only during acute attacks both from Government and private hospitals. About 74.7% were aware of and practising MMDP and attributed the source of awareness to PHC. In 2013, 52.2% received MMDP kits (soap, towel and a plastic basin and mug) once through respective PHCs.

Frequency and duration of acute dermato-lymphangio adenitis (ADLA) are considered as the indicators for the assessment of the impact of MMDP. However, filarial lymphoedema being a chronic disease with serious physical, social and psychological consequences due to disability and disfigurement, HRQoL, a multidimensional questionnaire based construct measuring patient's physical, psychological, social health domains and well being has been proposed by WHO as an additional indicator to assess the impact of morbidity management. The severity levels were scored by assigning a numerical value between 0 (worst quality) to 4 (best quality). The mean score of each domain was compared with the mean score of a normal individual without LF manifestation. It was observed that the higher the grade of lymphoedema, the lower is the mean score. A total of 59 patients out of 67 recruited were assessed for HRQoL. Physical domain is the most affected in all the grades. Physical, psychological, and social domains are severely affected in grade 4 (**Figure 1.1**).

During the process of patient recruitment for the study, a total of 260 lymphatic filariasis lymphoedema patients in cohort-1 villages whose lymphoedema grades were assessed in 2004 by VCRC and those received the MMDP package from the Government of TN there-after were reassessed in 2014. It was observed that, 46% of grade 1, 52% of grade 2 and 92% of grade-3 cases lymphoedema either reversed back to normal or remained in the same grade (**Figures 1.2–1.4**). Therefore MMDP practices under programme mode has yielded the desired results in prevention of disability, but grade 1 & 2 cases need additional medical attention to prevent further progression to higher grades. The patients in the

FIGURE 1.1 **Average severity score in different health domains in filarial lymphoedema patients (N = 59)**



	Physical function	Psychological function	Social function	Symptoms
Gr-1	13.75	22.5	22.5	10.25
Gr 2	13.82	18	22.52	9.17
Gr 3	11.64	16.16	19.88	9.08
Gr 4	9.71	10.14	14.42	8.71
Normal	44	48	32	20

FIGURE 1.2 Prevention of disability in Grade-1 lymphoedema patients (N=78)

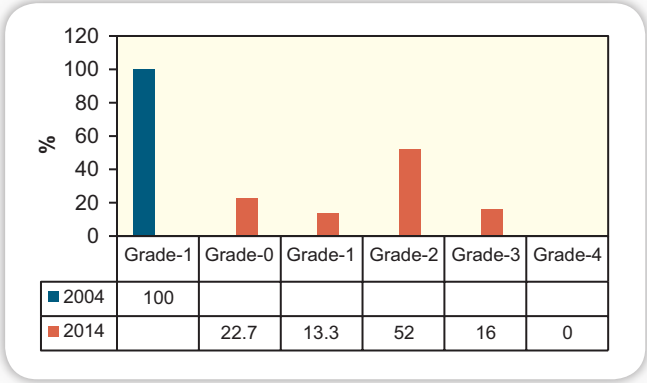


FIGURE 1.3 Prevention of disability in Grade-2 lymphoedema patients (N=106)

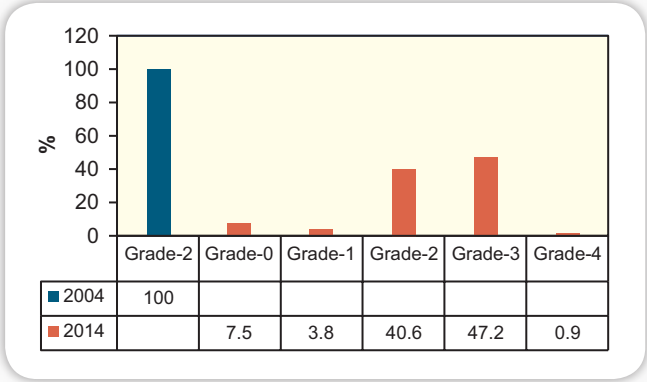
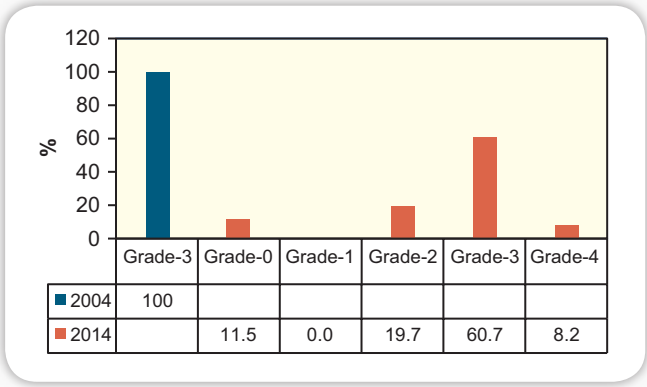


FIGURE 1.4 Prevention of disability in Grade-3 lymphoedema patients (N=61)



intervention group (cohort-3) are being trained in morbidity management procedures. The patients will be followed fortnightly for a period of one year for maintenance of good limb hygiene and impact will be assessed and compared with the pre intervention data. Data on impediments of MMDP are being collected at provider and user level from all the three study groups.

1.1.2 Assessment of impact of targeted semi-annual MDA in communities of antigen positive children in preventing resurgence during post MDA

IM 1402: Jul 2014 – Jun 2016

Krishnamoorthy K, Subramanian S, Vasuki V and Jambulingam P
Collaborator: Directorate of Public Health and Preventive Medicine, Chennai

Many filariasis endemic countries move closer towards the elimination goal and are in the process of evaluating the impact of the programme and deciding on stopping MDA. Transmission Assessment Survey is designed to assess antigenemia (Ag) prevalence among children who were born after the introduction of MDA. An evaluation unit (EU) is considered qualified when the number of children positive for Ag are below the critical level. If an EU fails in the subsequent TAS two years after stopping MDA, two more rounds of MDA is recommended. In India, MDA has been stopped in at least 12 out of 250 IUs, since the number of children positive for Ag were below the critical level. It is generally expected that transmission may cease consequent to the natural loss of these residual infections during post-MDA. However, the sites (clusters) with Ag-positive children indicate that local transmission was not totally interrupted by MDA even when the EU qualified for stopping MDA. These clusters may have potential to lead to resurgence and therefore warrants close monitoring during post-MDA period. Additional MDA in these communities (targeted) may be useful to liquidate the parasite load further to prevent the risk of resurgence of infection. Information on the usefulness/advantage of such targeted MDA in the residual foci to prevent resurgence compared to that without any intervention is important in developing appropriate strategies for post-MDA surveillance. In case of resurgence and failing TAS in the subsequent exercises, it would involve additional costs for two more rounds of MDA covering the entire evaluation unit and for further TAS exercises.

Objectives:

- To implement two more rounds of semi-annual MDA in communities with antigen positive children detected in TAS
- To compare the usefulness of targeted MDA in liquidating the parasite load in the population during post MDA surveillance.

In a workshop at the Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu, the results of pre TAS surveys were analysed jointly and identified ten districts qualified for TAS and an action plan was prepared in July 2014. TAS was conducted by the state health department in seven districts and all were qualified for stopping MDA.

Thanjavur district with over 2 million population was divided into two Evaluation units and school based TAS was carried out. In one of the evaluation units of Thanjavur district two clusters viz., Vazhkai subcentre with a population of 5696 in Kabisthalam PHC and Koodananal subcentre with a population of 7876 in Budalur PHC were found to have

antigen positive children. These two clusters are selected for the study. Surveys have been initiated to collect baseline data on Mf prevalence in the community and antigenemia prevalence in 6-7 years old children. Two rounds of MDA will be carried out at six monthly intervals in one of the clusters. Vector infection will be reassessed six months after two rounds of MDA by sampling 5000 female mosquitoes. Mosquitoes will be collected using gravid traps and screened for filarial infection using PCR. The results will be compared and the impact of additional two rounds of MDA will be assessed in terms of transmission parameters. Antigen survey among children in the age class 6-7 will be conducted to see the change in antigen prevalence following the additional intervention compared to the control.

1.1.3 Development of electrochemical based biosensor for detection of lymphatic filarial parasite, *Wuchereria bancrofti*, in vectors

EM 1209: Nov 2012 – Oct 2015

Hoti SL, Vasuki V (VCRC) and Senthil Kumar A (VIT University, Vellore)

The prototype of an electrochemical detector developed in the earlier project (Annual Report, 2010) needs to be further refined to a miniaturized version to reduce the volume of the analyte and also to make the devise user friendly and portable for the detection of *W. bancrofti* infection in vectors in peripheral areas.

Objectives:

- To optimize generation of shorter DNA fragments for use as probes and target molecules and prepare suitable chemically modified electrode
- To develop miniaturized version of cyclic voltammetry using screen printed electrodes.

Development of an improved asymmetric PCR for the generation of single stranded (ss) DNA and preparation of suitable chemically modified electrode (CME) to probe DNA hybridization process were reported earlier. The GCE/GO + Chit modified electrode provided a good platform for the hybridization of probe + target ssDNA. In order to get clear DNA hybridization detection signals, electrochemical impedence spectroscopy (EIS) measurement was chosen as an optimal technique. Typical EIS curve contains a semicircular part (at low resistance) and a linear line part (at high resistance). The semicircular part is responsible for electron-transfer reaction of the redox probe, while the linear line part is due to the diffusion of the redox probe. Higher the semi-circle indicates higher resistance (R_{CT}) for the electron-transfer reaction.

When EIS curves of various modified electrodes prepared using probe-1 and probe-2 with $Ru(bpy)_3^{2+}$ in pH 7 PBS for the DNA hybridization reaction were compared (**Table 1.1**), probe 1 modified electrodes showed significant variation (R_{CT}) in the EIS signals upon various modification procedures (**Figure 1.5**). Reproducibility of the electrode was found appreciable with comparable voltametric patterns when subjected to DNA hybridization process.

Further optimization of CME showed that 1pM of the probe concentration and 10 pM of the target ss-DNA were optimal for the electrochemical hybridization. Based on these optimal conditions, validation of real samples was carried out using the PCR amplicons of filarial parasite *Wuchereria bancrofti* (WB) as target DNA and *Brugia malai* (BM) & *Culex quinquefasciatus* mosquito (CQ) as mis match DNAs for the hybridization analysis. The voltammetric experiments showed good differentiation with target DNA against mismatch DNA suggesting the possibility to extend to proto-type electrochemical sensor. EIS also showed variation in the respective impedance signals of the real samples and were reproducible (**Figure 1.6**). This approach will be extended to portable proto-type sensor and validation of samples will be carried out and the project will be completed.

1.1.4 Development and validation of sampling strategies for xenomonitoring of infection in *Culex* vector by PCR as a surveillance tool for assessing post-MDA situation of lymphatic filariasis elimination programme

EM 1001: Apr 2010 – Dec 2015

Subramanian S[1], Sadanandanae C[1], Vasuki V[1], Abdul Khader MSM[1], Krishnamoorthy K[1] & Jambulingam P[1]

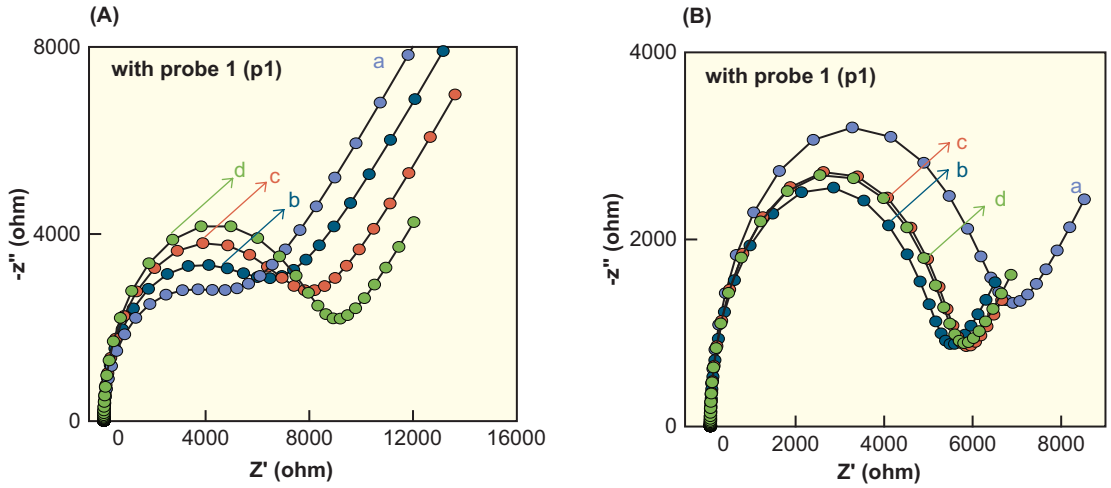
[1]Vector Control Research Centre, Puducherry, India, [2]Institute of Zoonoses and Vector Control, Department of Public Health, Hosur, Govt. of Tamil Nadu

Monitoring and epidemiological assessment of mass drug administration (MDA) and post-MDA surveillance are important components of LF elimination programme.

TABLE 1.1 Resistance (R_{CT} value) for the electron transfer reaction obtained for Probe 1 and Probe 2

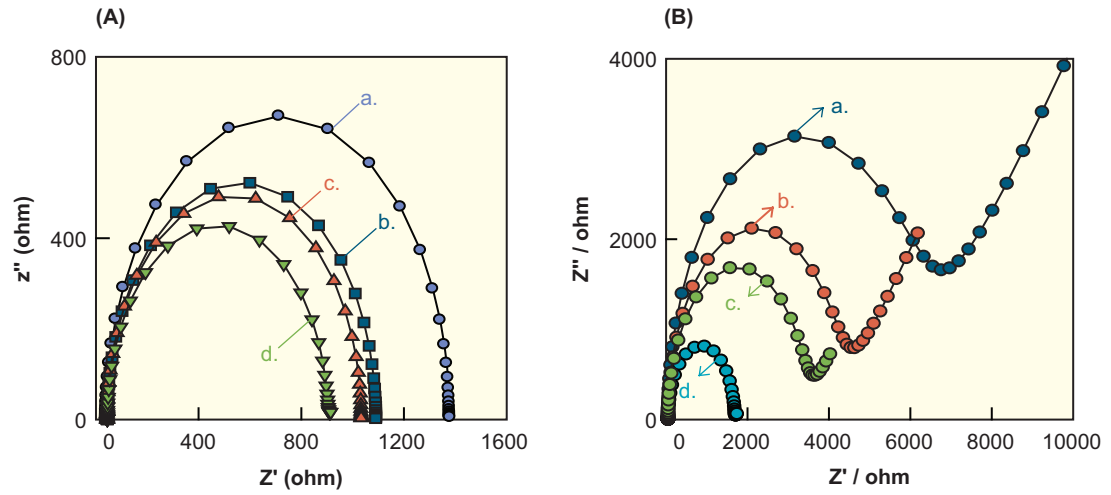
S.No.	Modified electrode with probe-1 (p_1)	R_{CT} value (KΩ)	Modified electrodes with probe-2 (p_2)	R_{CT} value (KΩ)
1	GCE/GO@Chit	4.48	GCE/GO@Chit	6.16
2	GCE/GO@Chit/p_1	5.68	GCE/GO@Chit/p2	4.98
3	GCE/GO@Chit/p_1-T	6.91	GCE/GO@Chit/p2-T	5.33
4	GCE/GO@Chit/p_1-M	7.96	GCE/GO@Chit/p2-M	5.26

FIGURE 1.5 Nyquist plots of 5mM $Ru(bpy)_3^{2+}$ using 0.5M KCl obtained at various modified electrode



[A] (a) GCE/GO@Chit; (b) GCE/GO@Chit/p_1; (c) GCE/GO@Chit/p_1-T and (d) GCE/GO@Chit/p_1-M
[B] (a) GCE/GO@Chit; (b) GCE/GO@Chit/p_2; (c) GCE/GO@Chit/p_2-T and (d) GCE/GO@Chit/p_2-M,
where T = Target ss-DNA and M = Mismatch ss-DNA

FIGURE 1.6 Nyquist plots of $Ru(bpy)_3^{2+}$ in 0.1M PBS obtained at various modified electrode



[A] (a) GCE/GO + Chit; (b) GCE/GO + Chit/p-DNA; (c) GCE/GO + Chit/p-T DNA and (d) GCE/GO + Chit/p-Mis DNA;
(Where mismatch is *Brugia malayi*)
[B] (a) GCE/GO + Chit; (b) GCE/GO + Chit/p-DNA; (c) GCE/GO + Chit/p-TDNA and (d) GCE/GO + Chit/p-Mis DNA;
(Where mismatch is *Culex quinquefasciatus*)

Programme success or failure can be monitored by measuring the changes in infection status in either the human or vector. This necessitates development of rapid surveillance tools and appropriate sampling strategies that could identify the low level of infection following multiple annual rounds of MDA. Detection of filarial parasites in mosquitoes indicates the existence of a reservoir of Mf in the human host while the presence of infective L3-stage larva signifies and quantifies transmission potential. PCR-based assays are found to be more rapid, sensitive and specific for detecting the presence of filarial infection in mosquitoes. These assays could be used for evaluating the impact of filariasis elimination programme and for post-MDA surveillance. However, its application in programme (in place of human sampling) requires sampling methods for mosquito collection and standardization of assays. Therefore the present study aims at addressing these issues based on field based observations.

Objectives:

- To evaluate a mosquito collection sampling strategy that can be used to assess the usefulness of vector infection monitoring by PCR as a surveillance tool for assessing post MDA situation

- To assess the usefulness of gravid traps for monitoring vector infection in relation to Indoor Resting collection by insecticide impregnated fabric traps
- To compare the long term changes in vector infection

The study was conducted in one of the PHCs (Ammapettai) in Thanjavur district, which had undergone eight rounds of MDA by 2010 and had two stages: assessing vector infection (i) following 8 rounds of MDA (i.e. in 2010) and (ii) two years after stopping MDA (i.e. in 2012). In each stage two independent surveys were carried out to collect mosquitoes by using gravid traps. Briefly the methodology involves collection, identification and preservation of mosquitoes in pools of 25 each for detecting infection by PCR assays. Mosquito sampling was done by fixing gravid traps in selected houses (following two-stage cluster sampling design) covering all the 33 villages / wards (clusters) of PHC as well as 17 streets (clusters) of wards (considered to be 'hotspots for LF transmission) in the same PHC. Using two different extraction procedures (crude DNA by VCRC and purified DNA by commercially available qiagen kit) filarial parasite DNA was extracted from parallel mosquito pools of same origin and the DNA was subjected to quantitative PCR assays.

In our earlier report, we have presented the results of qPCR assay done on crude DNA by VCRC method for stage 1 of the study. In this report, the results of qPCR assays based on DNA extraction by both VCRC and qiagen methods for stage 2 (Year 2012, two years post-MDA) of the study are presented by comparing the results with stage 1 (Year 2010, just after completion of 8 annual rounds of MDA). Also, the results of RT-PCR assay for detecting infective L3 larvae for stage 1 and 2 are compared.

Figure 1.7 and **1.8** show the results of qPCR assay based on DNA extraction by qiagen and VCRC methods for stages 1 and 2 of the study. In Stage 2, the vector infection rates (95% CI) by qiagen method in surveys 1 and 2 were 0.30% (0.16-0.51) and 0.24% (0.12-0.43) in the PHC clusters (**Figure 1.7**). The corresponding values for the 'hotspots' in the PHC are 1.55% (1.15-2.03) in survey 1 and 0.50% (0.30-0.77) in survey 2.

PCR assay results based on DNA extraction by VCRC method in stage 2 were 0.68% (0.45-0.97) and 0.46% (0.28-0.72) respectively in surveys 1 and survey 2 in the PHC areas and the corresponding values for 'hotspots' in the PHC are 0.57 (0.36-0.86) and 0.83% (0.56-1.18) respectively (**Figure 1.8**).

As observed in stage 1, vector infection rates are 2 to 5 times higher in the 'hotspots' than that from the entire PHC areas. Further, the infection rates both in the 'hotspots' and in the entire PHC areas declined significantly ($P < 0.05$) two year after stopping MDA (irrespective of the method of DNA extraction).

The 95% CIs for the two surveys suggest that while the estimates corresponding to VCRC-DNA extraction method are consistent between surveys in both PHC clusters and 'hotspots' (95% CI overlap with each other), such consistency between surveys are found only in PHC clusters but not in the 'hotspots' (95% CI do not overlap).

FIGURE 1.7 Comparison of vector infection rates based on qPCR assay using filarial parasite DNA extracted by qiagen method

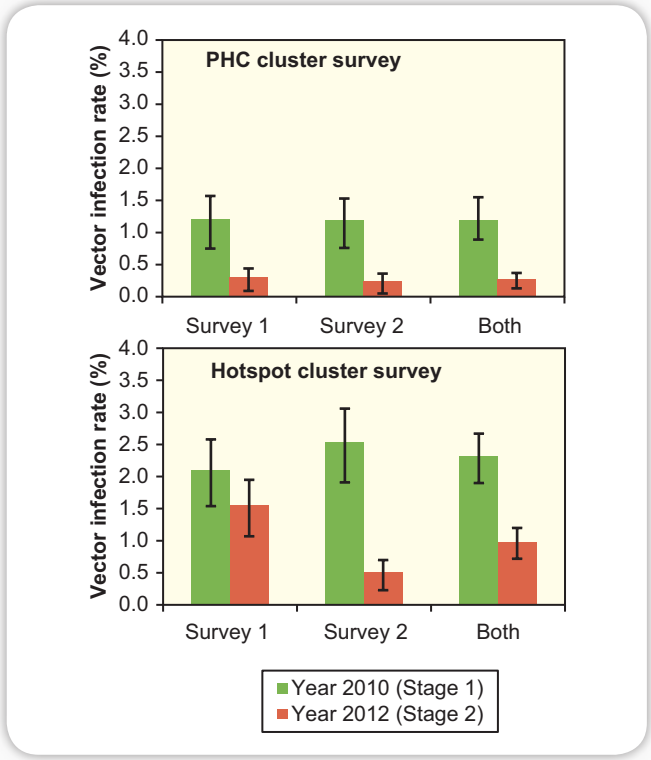
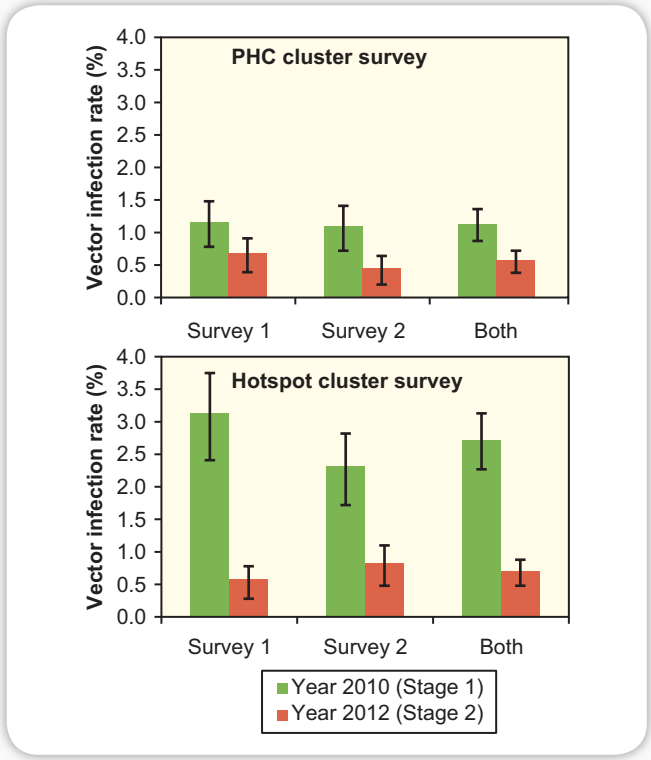


FIGURE 1.8 Comparison of vector infection rates based on qPCR assay using filarial parasite DNA extracted by VCRC method



A further comparison of these estimates with that derived by combining data from the two surveys in the PHC areas (0.27%; 95% CI: 0.17-0.41) and 'hotspot' areas (0.98; 95% CI: 0.76-1.24) showed that the estimates based on the two independent surveys are in agreement with the overall estimate from the respective areas (95% CIs overlap with that for overall estimate in the respective surveys).

The results in stage 2 of the study suggest that as observed in stage 1, the proposed mosquito sampling strategy of collecting 7 pools of 25 gravid females each from each of 33 clusters (aggregating to a total of 231 pools of at least 5000 gravid females) would suffice to assess the vector infection rates by PCR assays even when the infection rates in human and vectors are below the transmission threshold level of 0.5%.

In each stage, a total 207 pools were screen for assessing infectivity of the vector. The pool size varied from 5 to 25 in both stages. A total of 13 (6.3%) and 23 (11.1%) pools were found positive for L3-larave. Pool screen analysis showed that the infectivity rates were 0.26% and 0.51% immediately after 8 rounds of MDA in 2010 and 2 years after stopping MDA in 2012 respectively (**Table 1.2**). The 95% CIs suggest that the increase in infectivity rate two years after stopping MDA 2012 was not significant (95% CI overlaps).

Two sampling plans were simulated to assess whether sampling effort (sampling 300 households (HH) x 1 pool per HH instead of 150 HHs x 2 pools per HH) could be minimized without loss of statistical power. The results showed that both the pool positivity and the vector infection rates were comparable between the two plans (**Figure 1.9**). Further, it was found that only in less than 2% of the simulations both the results differ significantly between plans.

A sample of 210 pools (30 clusters x 7 pools) consisting of minimum 5000 gravid females is sufficient to assess the prevalence of infection in the vectors (0.25%) during post MDA. Simulation results showed that a sample of 300 pools by collecting 2 pools of 25 mosquitoes each from 150 households could be used to reduce the sampling effort instead of one pool from each of 210 or 300 households respectively.

Vector infection rates are consistent between surveys in both PHC and 'hotspot areas two years after stopping MDA (replicable).

The consistency of the estimates (vector infection) between surveys immediately after 8 annual round of MDA and two years post-MDA, and the ability to tract the declining trend in infection two years after stopping MDA suggest that the proposed two-stage cluster sampling protocol (village and households) can be used for the collection of *Culex* gravid females by gravid traps for monitoring vector infection and infectivity during post-MDA surveillance.

It is proposed to assess the risk of infection five years after stopping MDA (long term impact) in the study area which is qualified for certification.

FIGURE 1.9 **Comparison of simulated sampling plans by pool positivity and vector infection rates using data from all the 'PHC clusters'**

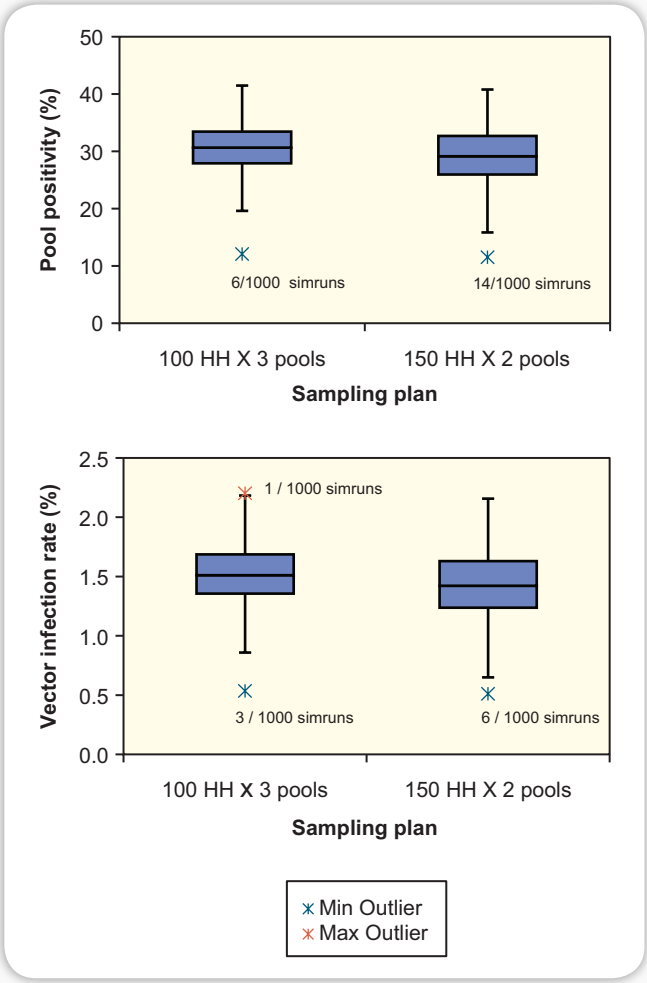


TABLE 1.2 **Results of RT-PCR assay for detecting filarial infectivity in *Culex* vector**

Stage (Year)	# pools screened	Pool size	# pools positive (%)	95% CI	Infectivity rate (%, 95% CI)
Stage 1 (2010)	207	25–25	13 (6.3)	3.0-9.6	6.8–15.4
Stage 2 (2012)	207	5–25	23 (11.1)	6.8-15.4	0.51 (0.31–0.78)

1.1.5 Field validation of sampling strategies for xenomonitoring of infection in *Culex* vector by PCR as a surveillance tool for lymphatic filariasis elimination programme

IM 1403: Jun 2014 – May 2016

Subramanian S, Krishnamoorthy K, Sadanandanae C, Vasuki V, Jambulingam P, and investigators from the Directorate of Public Health, Tamil Nadu, Puducherry, Goa

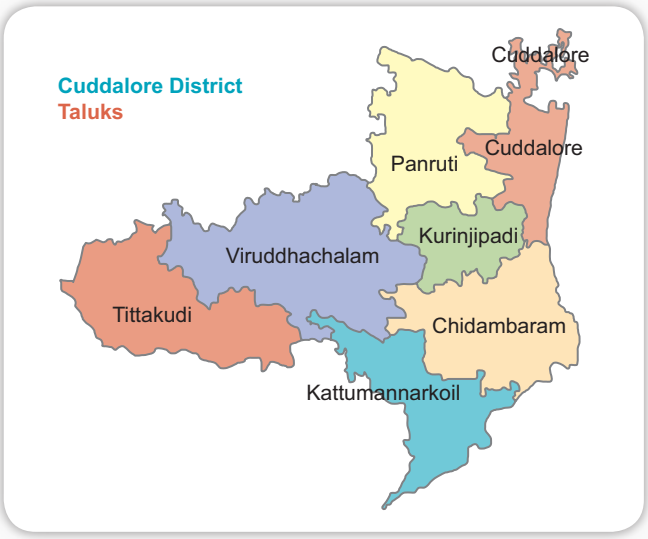
Currently, Transmission Assessment Survey (TAS) is recommended for making decision on stopping MDA and Post-MDA surveillance until certification of elimination of LF. TAS is based on detecting antigen in children (age-group: 6-7 years) to verify absence of transmission. The decision to stop MDA based on TAS and the subsequent TAS during post-MDA until certification involves use of ICT, which is expensive, and there are issues on the size of the evaluation unit it should encompass. Monitoring infection in vectors is appropriate when human infection levels are very low following the cessation of MDA. However, a difficulty in using filarial prevalence in mosquitoes for making decisions on stopping MDA or post-MDA surveillance is that no accepted threshold is available for vector infection. The VCRC has developed and tested a two-stage cluster design based sampling strategy for collecting vector mosquitoes and monitoring vector infection by PCR assay. The usefulness of xenomonitoring as an alternative to TAS during post MDA surveillance needs to be assessed in operational settings.

Objective:

- To assess the usefulness of xenomonitoring of vector infection as an alternative to TAS

As per the methodology, four implementation units (IU), namely, Cuddalore, Villupuram, Ariyalur and Puducherry were selected for the study. Of them, the last three qualified TAS and therefore MDA was stopped in these IUs. Steps were taken to initiate mosquito survey in the Cuddalore IU. The IU has seven taluks (Cuddalore, Chidambaram, Kurinchipadi, Pantruti, Kattumannarkoil, Tittakudi, and Virudhachalam) (**Figure 1.10**) with a total population of 2.4 million. Since the total population of the IU is above two million, the IU was divided into two evaluation units (EU1 and EU2) as per the WHO protocol for conducting TAS. The taluks Cuddalore, Chidambaram, Kurinchipadi and Pantruti formed EU1 (population: 1.4 million) and the taluks Virudhachalam, Tittakudi and Kattumannarkoil formed EU2 (population: 1.0 million). Of the two EUs in the IU, it was decided to select EU1 for the study. Details of population and number of households by village / ward (cluster) in each PHC / town in EU1 were obtained from the Census Department. For the purpose of mosquito survey, in stage 1 of the two stage (cluster and household) cluster sampling method, 30 clusters were selected. In stage 2, on an average 5 households (HH) per cluster were selected with probability proportional to size of the selected cluster for fixing gravid traps and in each household two pools of each with 25 *Culex* gravid female mosquitoes will be sampled (i.e. 30 clusters x 5 HHs per cluster x 2 pools per HH x 25 mosquitoes per pool = 7500 mosquitoes). Mosquito survey is in progress in the selected villages.

FIGURE 1.10 **Evaluation Unit 1 (4 Taluks) selected for study in Cuddalore district**



1.2.1 Effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to mass drug administration towards elimination of the lone foci of diurnally sub periodic *Wuchereria bancrofti* in Andaman & Nicobar Islands

EM 1305: Jun 2013 – May 2016

Shriram AN, (RMRC, Port Blair), Krishnamoorthy K, Amitabh De, Avijit Roy (Directorate of Health Services, Andaman & Nicobar Administration, Port Blair)

Nancowry group of Islands in Andaman and Nicobar islands, with a total population of 10733, is endemic for diurnally sub periodic *W. bancrofti*, transmitted by a day biting *Downsiomyia nivea* mosquito. It is the only focus of this infection in India and LF is an important public health problem among the tribal population in the islands. Preventive Chemotherapy (MDA) under LF elimination programme was launched in 2004 by the Directorate of Health Services, Andaman & Nicobar Administration. The mf prevalence was 10.5% prior to MDA. The results of Mf survey after six rounds of MDA in 2011 indicated persistence of the infection with an overall mf rate of 3.3% and with a maximum of 5.3% in one of the five islands. Therefore, it was felt necessary that supplementary measures such as Mass DEC fortified salt and vector control are implemented to accelerate the process of elimination. Considering the outdoor resting, diurnal biting habit and tree hole breeding behavior of the vector species, vector control including personal protection measures was considered not feasible. DEC medicated salt was considered as the potential supplementary option, particularly for the island situation where the influx of non-fortified salt can be controlled. Earlier, in a block level field trial in Tamil Nadu (population: 1.2 lakhs), it was demonstrated that one year mass distribution of DEC salt (0.2% w/w) as a supplement to five rounds of MDA was effective in reducing mf prevalence below 1% with no new infection among children (VCRC Annual Report 2010). Therefore, it was proposed to translate this strategy to eliminate the lone foci of diurnally subperiodic bancroftian filariasis in Andaman and Nicobar islands. This attempt is being made by the RMRC, Portblair and VCRC, Puducherry jointly with the Directorate of Health Services, Andaman & Nicobar Administration.

Baseline data were collected on microfilaria prevalence, antigenemia prevalence among children and salt usage pattern of the community in the five Nancowry group of islands. Besides contributing to the project design, the VCRC participated in collection of baseline data and analysis, particularly in collection and processing of samples for Antigenemia prevalence using ELISA.

Overall 1522 children were enumerated from 34 villages/hamlets spreading across the five islands for assessing the antigenemia status in the 2-4 (N = 858) and 6-7 (N = 664) age classes respectively. The number of children enumerated in 2-4 age class ranged between 95 (Nancowry) and 309 (Kamorta), while the children enumerated in 6-7age class ranged between 79 (Chowra) and 200 (Kamorta) respectively. Of these 1522 children enumerated, 642 (N = 436 in 2-4 and N = 206 in 6-7 age classes) were screened for assessment of antigenemia status. The overall coverage for antigenemia was 50.8% (2-4 age class) and 31% (6-7 age class) respectively. The coverages between islands varied between 44.0% (Chowra) and 61.1% (Nancowry) for 2-4 age class, while in the 6-7 age class the coverage ranged from 20.3% (Chowra) to 36.5% (Kamorta).

Overall, 14 (3.2%) and 6 (2.9%) children were positive for antigenemia in 2-4 and 6-7 age classes respectively. Prevalence of antigenemia in 2-4 age class varied between 0.0% (Teressa) to 5.9% (Chowra), whereas the prevalence in 6-7 age class ranged between 0.0% (Katchal) and 6.3% (Chowra) respectively. The current prevalence pattern of antigenemia indicates persistence of infection and evidence of recent transmission in Nancowry group of islands. Even in islands with less than 1% Mf prevalence, the antigenemia prevalence among children were more than 2%, indicating evidence for recent transmission.

Based on the results of Mf prevalence and antigenemia prevalence, the islands were grouped and randomized into two arms, one for intervention and the other for control (comparison).

We have approached Tamil Nadu Sal`t Corporation for DEC fortification and the Salt Corporation has responded positively. Our requirement of fortified salt has been estimated to about 30 metric tons for one year. Preliminary observations on stability of salt on storage in terms of concentration and quality of salt have been initiated. The intervention will be implemented as soon as the salt supply is made (**Table 1.3**).

TABLE 1.3 Details of the Baseline data in the study area

Arm	Island (Village)	Population	Mf prevalence (%)	Ag prevalence among children (6–7 years) (%)
Intervention (MDA + salt)	Teressa	1909	3.0	2.3
	Nancowry	1146	0.2	3.8
Comparison (MDA alone)	Chowra	1499	2.9	6.3
	Kamorta	3430	0.5	4.1

1.3.1 Tolerability, efficacy and operational feasibility of Artesunate Combination Therapy (ACT) (Artesunate – Sulphadoxine-Pyrimethamine): as 1st line anti-malarial drug for falciparum malaria control in a tribal area of Odisha state, India

EM 1207: Jul 2012 – Jun 2014

Das LK, Sahu SS, Krishnamoorthy N

Following the introduction of artmemisinin combination therapy (Artesunate-Sulphadoxine-Pyrimethamine) as 1st line of treatment for uncomplicated falciparum cases in malaria control programme by NVBDCP, the present study has been initiated in a stable falciparum area, Laxmipur CHC in Koraput district of Odisha State, to monitor the efficacy of this drug combination.

Objectives:

- Process of diagnosis and treatment of malaria at community level by health workers (ASHAs).
- Therapeutic efficacy of ACT (Clinico- parasitological response) at community level.
- Adverse Drug Events (ADE) with ACT, if any.

The study was carried out in Laxmipur upgraded primary health centre area with a population of 61,772 in Koraput district of Odisha state. Process of diagnosis and treatment of malaria by ASHAs was assessed through a structured pre-tested questionnaire interview. Therapeutic efficacy study was carried out following WHO 2008 Therapeutic efficacy protocol. Adverse drug events were monitored at village level daily for 7 days.

A total of 108 out of 116 ASHAs were interviewed. .Among the 108 ASHAS, all were trained and 81.48% were literate, 55 (50.92%) had less than 5 years of service experience, 73 (67.59%) had RDT kits with them at the time of survey and 83% could be able to perform the RDT. Among the ASHAS interviewed, 57.40% gave one time ACT for all 3 days.

A total of 115 villages were surveyed and 1794 fever cases were detected. Out of 250 *P.falciparum* cases detected, 93 were taken for the study following inclusion and exclusion criteria.

A total of 75 children out of 93 cases recruited completed the 28-day follow-up. It was observed that PCR uncorrected adequate clinical and parasitological response (ACPR) was in 90.7%, 1.3% early treatment failure (ETF) and 8% with late parasitological failure (LPF) of cases.

Pre and post treatment filter paper blood samples of 65 cases were examined for *Pf* species by PCR technique. Six cases (9.2%) and five cases (7.7%) showed persistence of parasite DNA on 7th and 14th follow-up respectively.

Abdominal pain was observed consistently and duration of this symptom remained up to 3 days after starting the treatment. However, the symptom was mild as it well tolerated by the patients (**Table 1.4**).

The results suggest that the ACT is well tolerated. The therapeutic efficacy of ACT on uncomplicated *Pf* infections indicates that ACPR was 90.7%, LPF in 8.0% and early ETF in 1.7% of cases. The major adverse effect was abdominal pain which lasted for up to 3 days after starting the treatment. However, the symptom was mild as it was easily tolerated by the study patients. Performance of ASHAs was satisfactory. However, regular replenishment of RDT kits and drugs is necessary. Training of ASHAs at regular interval will be crucial in malaria control in this area.

TABLE 1.4 Adverse drug events after ACT observed within 7 days of initiation of ACT therapy (N = 90)

At least one adverse event % (95% CI)	N (%)	Mean duration +SD (days)	Range-days (Min–Max)
Fever	28 (31.1)	0.68+1.02	1–4
Headache	28 (31.1)	0.8+1.11	1–4
Myalgia	19 (21.1)	0.39+0.70	1–3
Nausea	13 (14.4)	0.25+0.71	1–1
Vomiting	5 (5.6)	0.08+0.34	1–2
Abdominal pain	33 (36.7)	1.1+1.23	1–3
Flatulence	1 (1.1)	0.07+0.31	1–1
Diarrhoea	2 (2.2)	0.05+0.29	1–2
Difficulty in swallowing	2 (2.2)	0.03+0.18	1–1
Conjunctivitis	2 (2.2)	0.02+0.13	1–1
Nasal irritation	29 (32.2)	1.97+2.03	1–5
Others*	4 (4.4)	–	–

**Hepatitis-1, Chest pain-1, Cough-1, Leucoderma-1*

1.3.2 Comparative assessment of the efficacy of two rounds of indoor residual spraying with DDT 75% @ one g/m² and DDT 50% @ one g/m² against, *Anopheles fluviatilis,* the malaria vector in Odisha State

EM 1301: Jun 2013 - Nov 2014

Gunasekaran K, Sahu SS, Subramanian S, Behera KP and Pradhan MM**
**Odisha State Health Department*

DDT, the most inexpensive and common insecticide used for malaria and kala-azar control, is available in two formulations, WDP 50% and WDP 75%. Although, DDT WDP 75% is generally considered as the cost effective high performing formulation for indoor residual spraying (IRS) with long lasting residual properties, information on the comparative efficacy of the two formulations in Indian conditions is essential to choose DDT 75% for IRS replacing DDT WDP 50%. Hence, this proposal to assess the comparative efficacy of indoor residual spraying of DDT WDP 75% @ one gm/m2 with that of DDT WDP 50% @ one gm/m2

against *Anopheles fluviatilis*, the primary vector of malaria, in the selected endemic areas of Odisha State.

Objectives:

General

- To evaluate the comparative efficacy of indoor residual spraying of DDT WDP 75% @ one gm/m^2 with that of DDT WDP 50% @ one gm/m^2 against the malaria vector, *An. fluviatilis*.

Specific

- To evaluate the impact of spraying DDT WDP 75% over DDT WDP 50% on the vector in terms of reduction in abundance, human blood index and survival of the vector.
- To determine the residual effect of the two DDT formulations on sprayed surfaces and,
- To assess the of quality of spraying, coverage and influence of wall smearing etc. on the effectiveness of residual spraying of both the formulations of DDT.

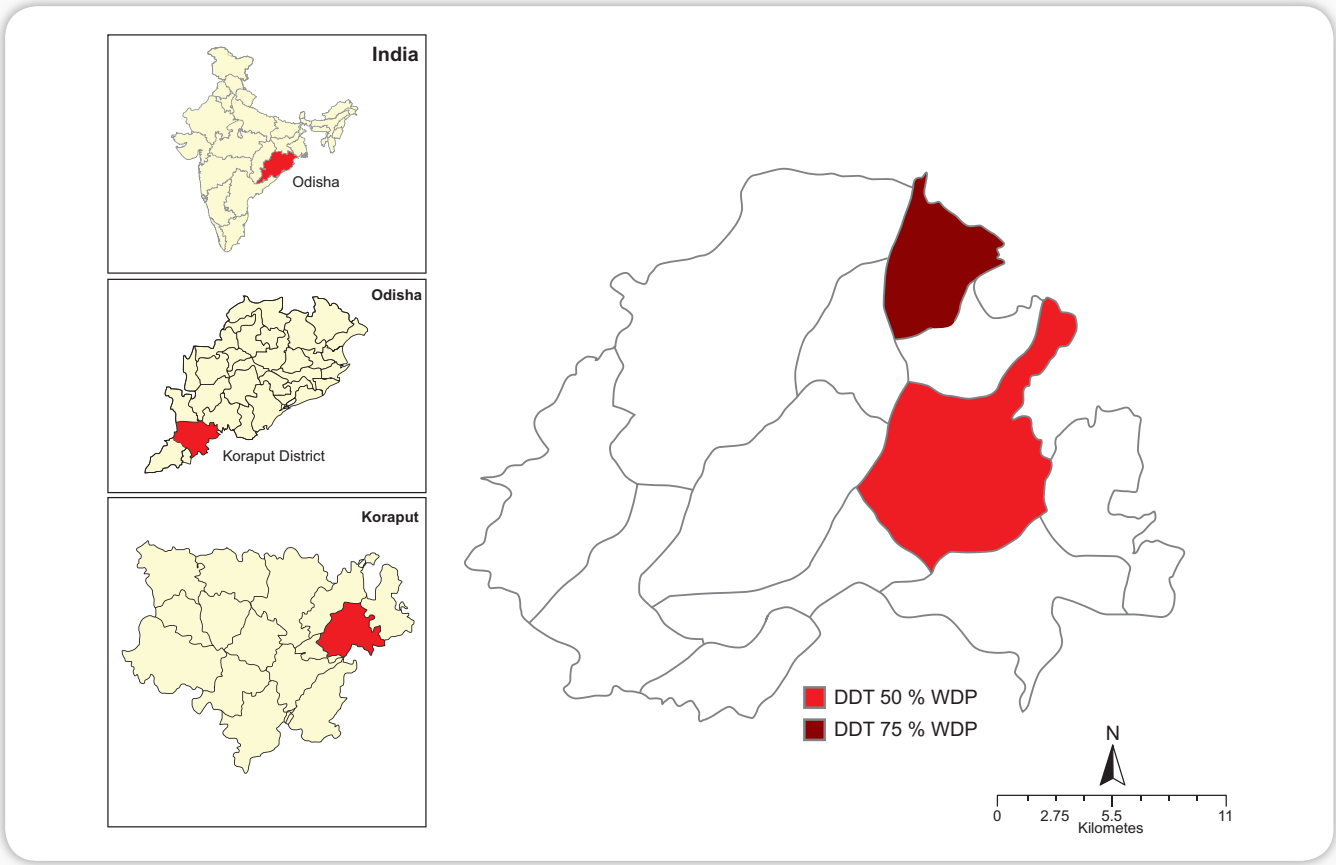
This intervention study has two arms, one with residual spraying of DDT WDP 50% @ one gm/m^2 in Kumbhari SC (9 villages) and the other with DDT WDP 75% @ one gm/m^2 in Jogipaluru SC (10 villages) of Narayanpatana community health centre (CHC) of Koraput district in Odisha State (**Figure 1.11**). DDT WDP 50% is currently in use for indoor residual spraying (IRS) for malaria control.

During the 1st round of spraying, overall room coverage was 83.4% (n = 1,425) in Kumbhari SC (74.7%–98.3% in different villages) and 86.9% (n = 1,473) in Jogipaluru SC (77.7%–99.1%). During the 2nd round, the coverage was 75.6% (n = 1467) in Kumbhari SC (70.3%–87.9%) and 80.1% (n = 1572) in Jogipaluru SC (70.4%–99.1%). The coverage was relatively lesser during the 2nd round than the 1st round in both the SCs as the 2ndround was delayed and coincided with the paddy harvesting time. Thus, some villagers, despite advanced information, locked their houses and went to fields for harvesting when the spray team visited their village.

Mud plastering of walls is a common practice in the study villages and by this practice people do mud-plastering sprayed walls also. After 1st round of spraying, the proportion of rooms with sprayed walls mud plastered gradually increased and at three months post-spraying, i.e. prior to 2nd round, the proportion reached > 90% in both the arms.

After the 2nd round of spraying, within a fortnight, 2.7% and 3.3% of the sprayed rooms were found mud-plastered in the 50% and 75% arm, respectively. Thereafter, there was a gradual increase in the mud-plastered proportion and by 5thmonth it reached 79.9% and 55.8%, respectively. Within another fortnight, i.e. at 5.5 months post-spraying, almost all the sprayed rooms (100% and 98%, respectively) were found mud-plastered in both the arms and this fast mud-plastering coverage was reportedly due to 'Chaitra parabo' an important local festival celebrated by the villagers.

FIGURE 1.11 **Map showing the two sub-centres, Jogipaluru (DDT 75%) and Kumbhari (DDT 50%) in Narayanpatana CHC of Koraput district, Odisha State, selected for the evaluation**



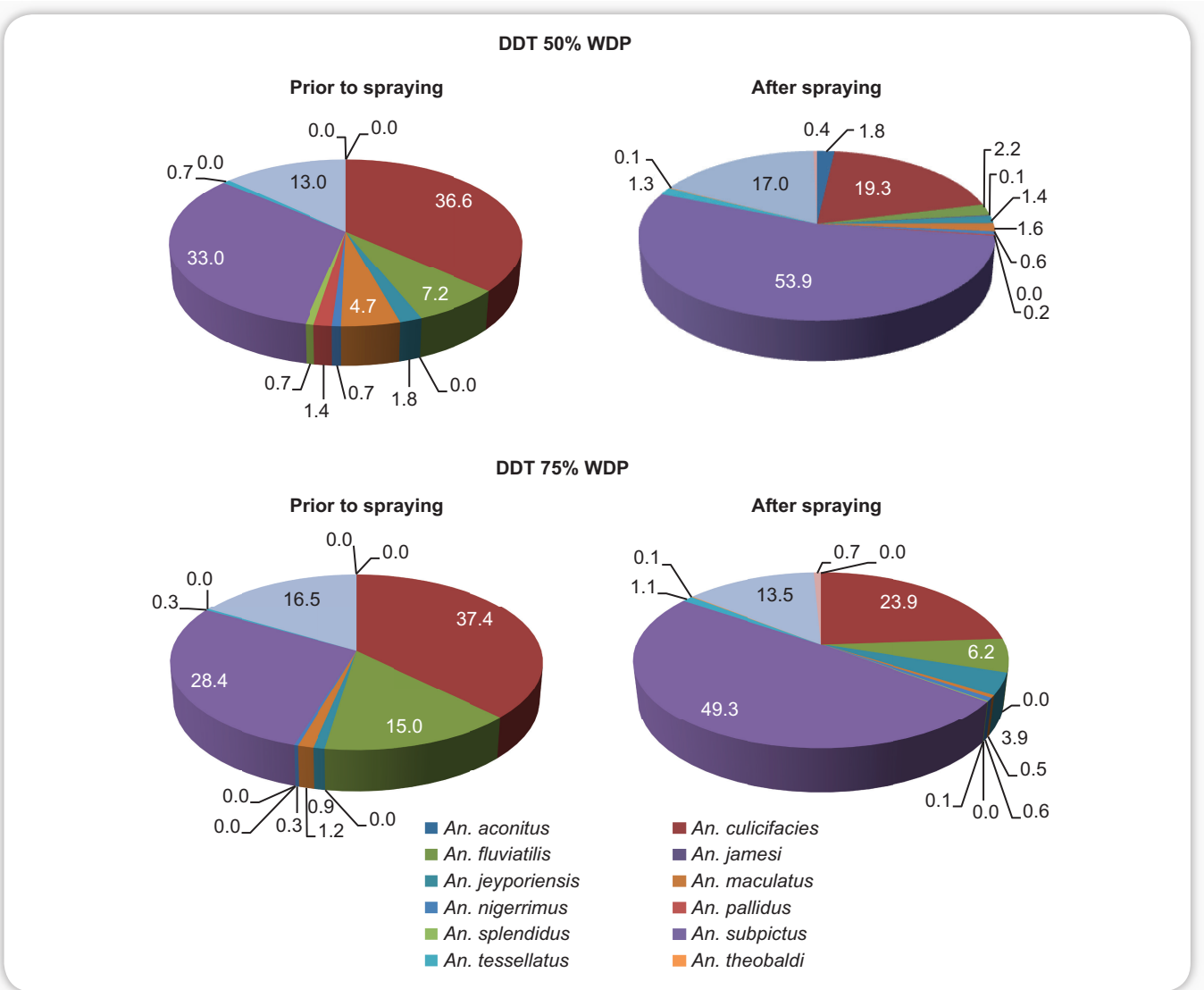
In total 2260 anophelines of 14 species were collected from the two arms. This included *An. fluviatilis and An. culicifacies,* the recognized vectors of malaria and *An. aconitus, An. jeyporiensis, An. maculatus* and *An. varuna*, the known vectors of secondary importance in India. Among the anophelines, the most abundant one was *An. subpictus* (46%) followed by *An. culicifacies* (26%), *An. vagus* (15%) and *An. fluviatilis* (6%).

In the 50% arm, prior to spraying the relative proportion of *An. fluviatilis* was 7.2% (n = 276) and after two rounds of spraying it was reduced to 2.2% (n = 829). Similarly, in the 75% arm there was a reduction from 15% (n = 334) to 6.2% (n = 821). Similar reduction in relative proportion of *An. culicifacies* (36.6% to 19.3% and 37.4% to 23.9%, respectively) was observed in both the arms after two rounds of spraying (**Figure 1.12**). The reduction in the relative proportion of the two vector species in the two arms could be attributed to the effect of spraying.

Prior to spraying, the per man hour density indoors (PMDI) of *An. culicifacies* in human dwellings of six index villages in Kumbhari SC (DDT 50%) varied from 0.2 to 5.2 with an average (± SD) of 2.7 ± 2.2 and in Jogipaluru SC (DDT 75%), it varied from 0.0 to 5.0 with an average of 3.3 ± 1.8; the average PMDI was not different between the two arms (F = 0.257, p = 0.623). After the 1st round of spraying, the average (± SD) PMDI was 2.4 ± 1.4 in the 50% arm and it was 3.2 ± 2.9 in the 75% arm. After the 2nd round, the corresponding values were 0.5 ± 0.7 and 0.3 ± 0.4. The low density in both the arms could be due to the seasonal effect (cold season) [**Figure 1.13 (a)**]. The PMDI from pre- to post-spray period were not significantly different between the two arms (Two-way ANOVA, interaction effect: F = 0.48, P = 0.6).

Before spraying, the average PMDI of *An. fluviatilis* was 0.17 ± 0.14 (range: 0.0 to 0.3) in Kumbhari SC (50% arm) and 0.18 ± 0.27 (range: 0.0 to 0.7) in Jogipaluru SC (75% arm); the PMDI did not differ significantly between the two arms (F = 0.018, p = 0.896). After the 1st round of spraying, the PMDI was reduced to '0' and maintained

FIGURE 1.12 **Relative proportions of *Anopheles* species, including *An. fluviatilis* and *An. culicifacies,* collected prior to and after two rounds of spraying in 50% and 75% arms**



almost at '0' level up to four months (up to week 21) i.e. up to just before the 2nd round of spraying in both the arms. After the 2nd round, the PMDI remained at '0' level up to five and half months (up to week 43) in both the arms [**Figure 1.14 (a)**], indicating both the DDT formulations produced a good and comparable impact on indoor resting density of *An. fluviatilis*.

Overall, in both the arms, the number of *An. culicifacies* collected with light traps in human dwellings was poor. The per trap-night density (PTD) ± SD of *An. culicifacies* in the 50% arm was 0.04 ± 0.09 prior to spraying and after 1st round of spraying the PTD was 0.05 ± 0.11. The corresponding values in the 75% arm were 0.06 ± 0.05 and 0.11 ± 0. Thus, no impact of spraying was reflected from the PTD of *An. culicifacies*. After the 2nd round of spraying, the PTD in both the arms was '0' up to April 2014 (week 43), which was due to the seasonal effect [**Figure 1.13 (b)**]. The relative reduction in PTD after spraying between the two arms was not significantly different (two-way ANOVA, interaction effect: F = 0.5, P = 0.6).

In the case of *An. fluviatilis*, both the DDT formulations significantly brought down the PTD after 1st round of spraying; in the 50% arm from 0.20 ± 0.16 to 0.01 ± 0.03 and in the 75% arm from 0.40 ± 0.37 to 0.08 ± 0.14 ($p < 0.05$ by one way ANOVA). After the 2nd round, the PTD remained significantly at a lower level ($p < 0.05$, compared to the density before spraying) with an average of 0.04 ± 0.07 and 0.01 ± 0.03 in the 50% and the 75% arm, respectively [**Figure 1.14 (b)**]. The relative reduction of PTD after spraying was not different between the two arms (two-way ANOVA, interaction effect: F = 2.25, P = 0.12), indicating a comparable effect of the two formulations in terms of reducing *An. fluviatilis* density.

The relative changes [**Figure 1.13 (c)**] in the per man hour density outdoors (PMDO) of *An. culicifacies* after spraying was not significantly different between the two arms (two-way ANOVA, interaction effect: F = 2.63, P = 0.08).

Prior to spraying, the PMDO of *An. fluviatilis* was 0.06 ± 0.13 in the 50% arm and 0.70 ± 0.58 in the

FIGURE 1.13 **Density of *An. culicifacies* prior to and after indoor residual spraying in Kumbhari and Jogipaluru SC with DDT 50% and DDT 75% formulations, respectively: (a) Per man-hour resting density indoors (PMDI), (b) Per trap-night density indoors (PTD) and (c) Per man-hour resting density outdoors (PMDO)**

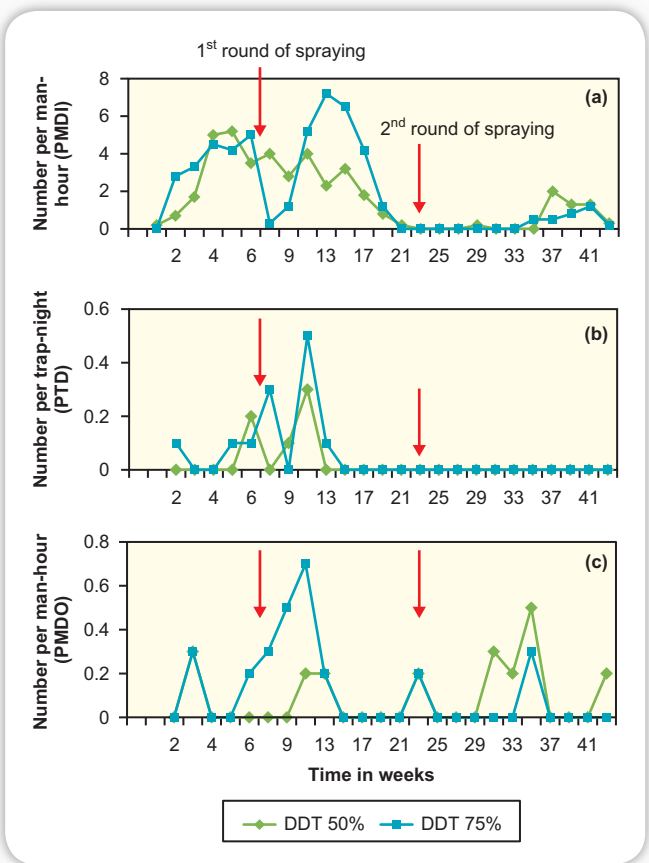
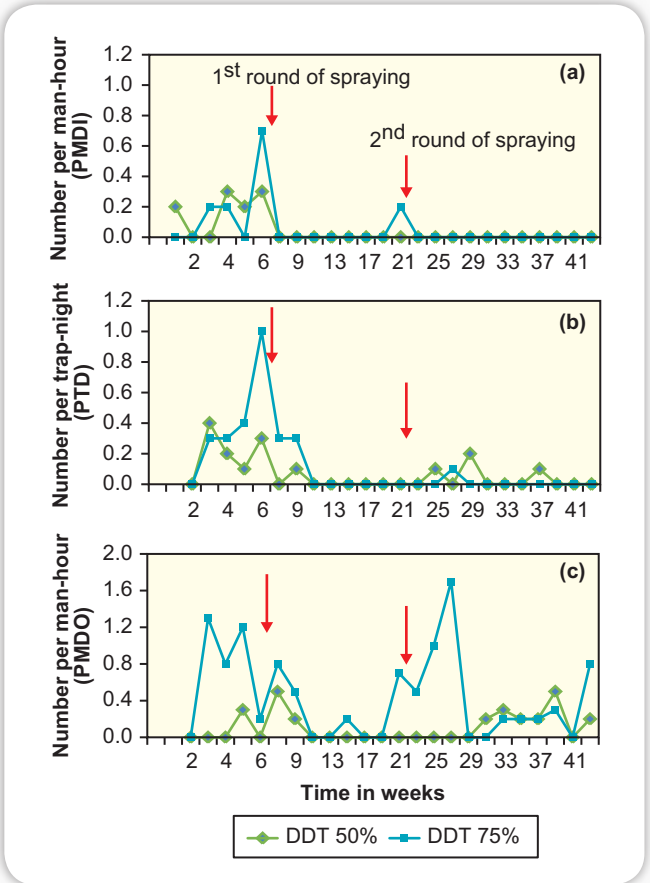


FIGURE 1.14 **Density of *An. fluviatilis* prior to and after indoor residual spraying in Kumbhari and Jogipaluru SC with DDT 50% and DDT 75% formulations, respectively: (a) Per man-hour resting density indoors (PMDI), (b) Per trap-night density indoors (PTD) and (c) Per man-hour resting density outdoors (PMDO)**



75% arm; the PMDO was not significantly different ($p > 0.05$). After the 1st round of spraying, the PMDO was 0.09 ± 0.18 and 0.28 ± 0.34 in the 50% and 75% arm, respectively. The corresponding values after the 2nd round of spraying were 0.15 ± 0.16 and 0.45 ± 0.53 [**Figure 1.14 (c)**]. The relative changes in the PMDO of this species after spraying between the two arms was not significantly different (two-way ANOVA, interaction effect: $F = 1.2$, $P = 0.3$).

The logistic regression analysis showed that the relative reduction in parous rate of *An. culicifacies* after spraying did not differ significantly between the two arms ($p = 0.13$). The parous rate of *An. fluviatilis* before spraying was 50% ($n = 6$) in both the arms. After spraying, there was almost nil collection in human dwellings and hence parous rate could not be calculated.

The fully fed female mosquitoes collected from indoor and outdoor resting sites were used for blood meal analysis. Prior to spraying, human blood index (HBI) of *An. fluviatilis* was not different between the two arms; 0.92 ($n = 13$) in the DDT 50% and 0.97 ($n = 30$) in the DDT 75% arm ($c^2 = 0.4$, $P = 0.5$). After the two rounds of spraying, the HBI of this species was significantly reduced in both the arms, 0.33 ($n = 6$) in the 50% ($c^2 = 7.3$, $p = 0.007$) and 0.43 ($n = 14$) in the 75% arm ($c^2 = 16.9$, $p < 0.01$). However, the relative reduction in HBI of this vector species after spraying between the two arms was not different (logistic regression, Wald statistic = 0.4, $P = 0.5$). Similarly, the relative changes occurred in HBI of *An. culicifacies* after spraying between the two arms was not significantly different (logistic regression, Wald statistic = 0.04, $P = 0.8$).

The results of the PCR assay for vector infection in both the arms before and after 1st round of spraying are given in **Table 1.5**. The maximum likelihood estimate (MLE) of infection rate of *An. culicifacies* was 3.6% before spraying and 0.9% after spraying in the 50% arm and the corresponding values were 0% and 1.2% in the 75% arm. The infection rate of *An. fluviatilis* was 7.9% before spraying and after spraying it was 7.1% in the 75% arm, whereas in the 50% arm, the respective rates were 0% and 21%. The vector infection rates in both the vector species was not significantly different between the two arms after spraying (95% CI overlap for all comparisons).

Before spraying, cone-bioassays (**Figure 1.15**) carried out on wall surfaces in both the arms showed zero mortality of *An. fluviatilis* ($n = 20$) and *An. culicifacies* ($n = 300$) confirming absence of insecticide deposit on the walls. Two weeks after 1st round of spraying, the mortality of *An. culicifacies* was 10.7% ($n = 150$) and 29.3% ($n = 150$) on DDT 50% and DDT 75% sprayed surfaces, respectively. Since, *An. culicifacies* was a DDT resistant species and *An. fluviatilis* was not available in adequate numbers, subsequent bioassays were carried out using DDT susceptible *An. stephensi*. After one month of spraying, the mortality of *An. stephensi* was 98.4% ($n = 190$) against DDT 50% and 99.5% ($n = 190$) against DDT 75%. Three months after spraying, while DDT 75% caused a mortality of 97.4% ($n = 420$), the mortality decreased to 71.0% ($n = 210$) against DDT 50%. After three months of spraying against the mud-plastered surfaces, the mortality was 80.8% ($n = 255$) with DDT 75% and 47.9% ($n = 390$) with DDT 50% [**Figure 1.16 (a) & (b)**].

After 2nd round of spraying, the mortality against the sprayed surfaces with the two DDT formulations was 100% ($n = 450$) up to 45 days. At 3.5 months post-spraying, the mortality reduced to 81.3% ($n = 450$) with DDT 50% and 90.7% ($n = 450$) with DDT 75%. The respective mortality after 4.5 months of spraying, was 79.3% ($n = 420$) and 85.1% ($n = 450$). Thereafter, bioassays were not done since all the rooms of the sprayed houses were completely mud-plastered in both the arms. At 5.5 months after spraying, against one time mud-plastered surfaces, the mortality was 44% ($n = 450$) with DDT 50% and 57.3% ($n = 450$) with DDT 75% [**Figure 1.17 (a) & (b)**].

The bioassay was also done with *An. fluviatilis* on two occasions after 2nd round. At 20 days and three months post-spraying, the mortality against DDT 50% was 100% ($n = 20$) and 92.3% ($n = 65$), respectively, and against DDT 75% the mortality was 100% ($n = 20$ & 65) on both the occasions [**Figure 1.17 (a)**].

In total, 156 adult household members and 58 pregnant women in the 50% arm and 164 adult members and

TABLE 1.5 **Maximum likelihood estimation (MLE) of infection rate in vector mosquitoes before and after 1st round of spraying as obtained from PCR assay results**

Test particulars	*An. culicifacies*				*An. fluviatilis*			
	DDT 50%		DDT 75%		DDT 50%		DDT 75%	
	Before spray	After spray	Before spray	After spray	Before spray	After spray	Before spray	After spray
No. of mosquitoes	59	112	72	166	12	5	42	15
No. of pools	13	24	15	36	5	2	11	6
No. of pools +ve	2	1	0	2	0	1	3	1
Infection rate (%) (95% CI)	3.62 (0.65–11.79)	0.89 (0.05–4.28)	0 (0.0–4.59)	1.22 (0.22–3.96)	0 (0.0–20.69)	20.98 (1.34–80.69)	7.92 (2.16–20.67)	7.09 (0.14–32.49)

FIGURE 1.15 **Preparedness by the people for getting their houses sprayed with DDT (a), a sprayed wall (b), a house with a mud-plastered wall (c), scratching mud sample from a sprayed wall for DDT residue analysis (d) and cone-bioassay on sprayed surfaces (e & f)**

50 pregnant women in the 75% arm were interviewed, over a period of 5.5 months, for their perceived side-effects, if any, after spraying their houses. In addition, 16 spray-men in each of the two arms were interviewed during spraying and the next day of spraying. Except the report of itching and headache by one spray-man in the 50% arm and nausea and headache by one pregnant woman in the 75% arm, none reported any side effect. Moreover, those who reported such side effects recovered within 12 to 24 hours.

Various parameters were considered to study the difference between the two DDT formulations in terms of ease of application (**Table 1.6**). Except preparation of the mixture (spray solution), there were no differences between the two formulations.

The evaluation is continued.

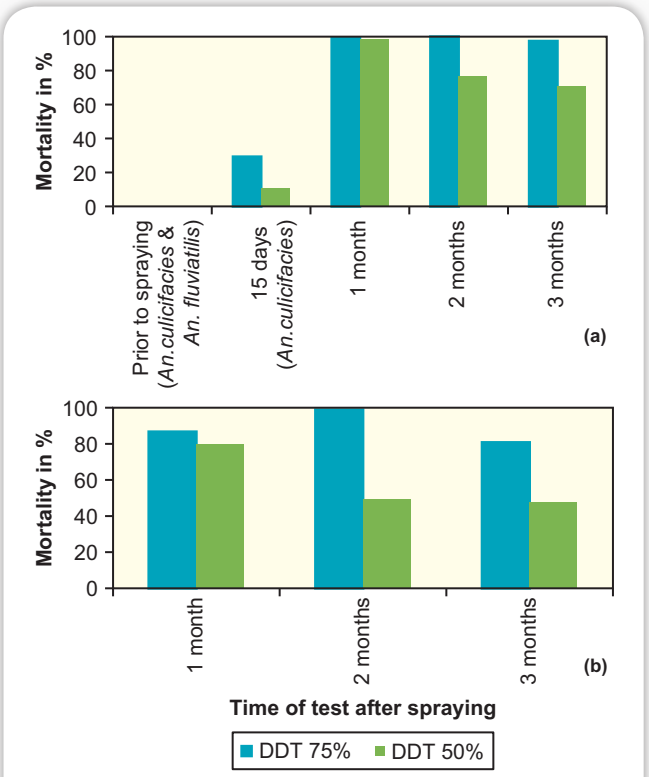
1.3.3 Comparative assessment of the impact of combo vector control [long lasting insecticide treated nets (LLIN) plus indoor residual spraying (IRS)] versus single measure (only LLIN or IRS) on malaria transmission in Koraput district of Odisha State

EM 1406: Nov. 2014 – Oct 2018

P. Jambulingam, K. Gunasekaran, S. S. Sahu, S. Subramanian, L. K. Das, K. P. Behera and Swati Kumari* (*Odisha State Health Department)*

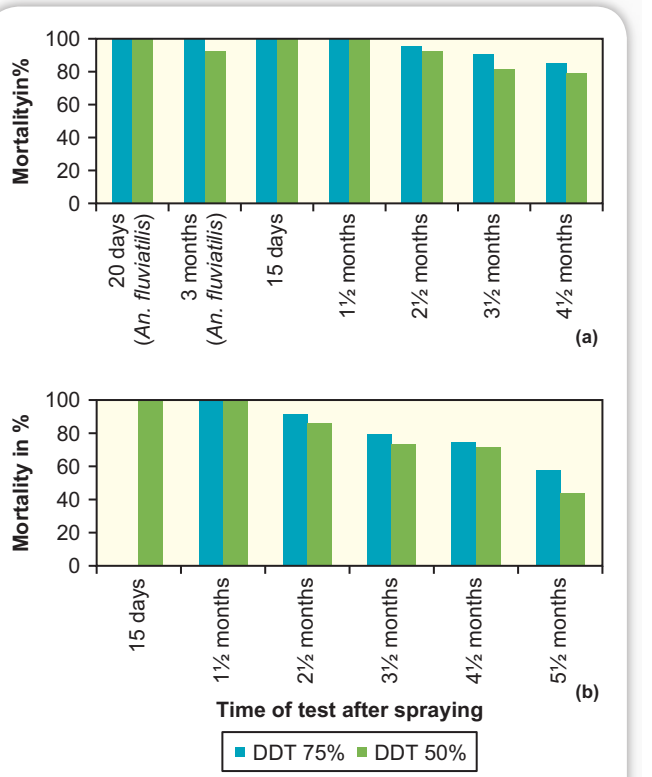
Though IVM entails optimal use of a range of interventions, separately or in combination in order to achieve cost-effective control of malaria and reduce reliance on any single

FIGURE 1.16 Mosquito mortality in bioassays done on sprayed surfaces (a) and on sprayed but mud-plastered surfaces (b) after 1st round of spraying



*Unless indicated, the mosquito species used for bioassays was **An. stephensi***

FIGURE 1.17 Mosquito mortality in bioassays done on sprayed surfaces (a) and on sprayed but mud-plastered surfaces (b) after 2nd round of spraying



*Unless indicated, the mosquito species used for bioassays was **An. stephensi***

intervention, there is no data available in the country on the impact of LLIN + IRS versus only LLIN or IRS on *fluviatilis* transmitted *falciparum* malaria. This proposal is to devise strategies for the optimal use of these two measures in combination (Arm 1) or singly (Arm 2, only LLIN & Arm 3, only IRS) through integrated approach and to make a comparative assessment of the interventions on mortality and morbidity due to *fluviatilis* transmitted *falciparum* malaria.

Objectives:

- To identify operational and technical issues relating to the implementation of combo-vector control (LLIN + IRS, Arm 1) versus single measure (only LLIN, Arm 2 or only IRS, Arm 3) for malaria control and optimize their use.
- To assess the relative impact of the three arms in terms of reduction in abundance (resting density), survival, human blood index (HBI) and infection of the vector species and malaria incidence and mortality due to malaria.

The study is carried out in Laxmipur CHC (sub-centres: 16, villages/hamlets: 174, population: 66,935) and Dasmanthpur CHC (sub-centres: 24, villages/hamlets: 290, population: 78,125) of Koraput district, Odisha State. In Laxmipur CHC, 86 villages under eight sub-centres (SCs) with a population of 32,966 (78.3% Kondho tribe) were kept in Arm 1 and 88 villages under 8 SCs with a population of 33,969 (52.6% Kondho tribe) were included under Arm 2. Arm 3 had 99 villages under 7 SCs with a population of 25,616 (33.2% Paraja tribe) in Podagada sector of Dasmanthpur CHC. The two CHCs have been highly endemic for malaria, *P. falciparum* being the predominant constituting >85% of the total cases. Of the 16 SCs in Laxmipur CHC, the State implements combo-vector control, LLIN (@ one net for 2.5 persons) + IRS (two rounds using DDT) from July-August 2012 in eight SCs recording higher incidence of malaria (Arm 1) and in the remaining eight SCs only distribution of LLIN (Arm 2). In the seven SCs of Dasmanthpur CHC, two rounds of IRS with DDT is continued (Arm 3).

The anophelines collected from the 23 study villages during the period from July 2012 to September 2014 included *An. fluviatilis and An. culicifacies,* the recognized primary vectors of malaria. The range of density {number per man-hour (PMD)} of the two vector species recorded in different months in human dwellings, cattle sheds and outdoors in the three arms are given in the Table 1. In all the three arms, the PMD of *An. culicifacies* was higher during rainy (July-October) than winter months (November-February). In contrast, the PMD of *An. fluviatilis* was lower during rainy months and higher during winter in all the three arms (**Table 1.7**).

TABLE 1.6 Ease of application of two DDT formulations, 75% WDP and 50% WDP, for indoor residual spraying

S.No.	Application	50% DDT	75% DDT	Difference
1	Mixture	1 kg DDT + 10 ltrs of water	676gm of DDT + 10 ltrs of water	Yes
2	Preparation of spray suspension	Uniform stirring	Uniform stirring	No
3	Pump used	Stirrup pump	Stirrup pump	No
4	Dosages	1gm/m^2	1gm/m^2	No
5	Man power used in each spray squad	5 FW + 1SFW = 6persons	5 FW + 1SFW = 6persons	No
6	Coverage per squad	Each squad covered 60 to 80 houses in plain and 50 to 60 houses in hilly area in a day	Each squad covered 60 to 80 houses in plain and 50 to 60 houses in hilly area in a day	No
7	Spray operation	One man operated the pump and the other man sprayed	One man operated the pump and the other man sprayed	No
8	Nozzle discharge rate	740 to 850 ml per minute	740 to 850 ml per minute	No
9	Spray lance	Kept 45 cm or 18 inches away from the wall surface	Kept 45 cm or 18 inches away from the wall surface	No
10	Spray swath	Parallel	Parallel	No
11	Application of Swath	Spray was applied in vertical swath of 53cm or 21inches wide	Spray was applied in vertical swath of 53cm or 21 inches wide	No
12	Successive Swath	Overlapped by 7.5 cm (3 inches)	Overlapped by 7.5 cm (3 inches)	No
13	Spray motion	Spray was done from roof to floor using downward motion	Spray was done from roof to floor using downward motion	No
14	Strokes of pump	20 to 26 strokes/min	20 to 26 strokes/ min	No
15	Plunger movement	10 to 15 cm	10 to 15 cm	No
16	Types of spray	Uniform	Uniform	No

TABLE 1.7 Resting density of the vector species in the three habitats and the three arms

	Resting density (Number per man-hour (PMD) range					
	An. culicifacies			*An. fluviatilis*		
Habitat	LLIN + IRS	Only LLIN	Only IRS	LLIN + IRS	Only LLIN	Only IRS
Human dwellings (HD)	0–1.6	0–1.1	0–0.3	0–0.9	0–0.3	0–0
Cattle sheds (CS)	0.4–19.5	0–15.6	2.0–12.4	0–5.1	0–3.0	0–0.4
Outdoors	0–0.5	0–0.2	0–0.1	0–0.8	0–0.4	0–0.1

In Arm 1, out of 147 blood meals of *An. fluviatilis* analysed, only 13.6% was positive for human blood, showing a lower HBI (0.14). In Arm 2, 21% (n = 81) was found positive for human blood, also showing a lower HBI (0.21). In Arm 3, blood meal analysis could not be done as the number of *An. fluviatilis* collected was too low.

After 20 months of field use, the bioassays done with *An. stephensi* on 12 nets from six randomly selected villages showed 100% mortality indicating full bio-availability of the insecticide. The LLIN use rate varied between 47.1% in summer to 67.7% in winter.

In all the three arms, *P. falciparum* was predominant constituting 95.4% (including the mixed infections with *Pf*) and the remaining 4.6% was due to *P. vivax* infection. There was a wide variation in monthly parasite incidence (MPI) recorded in the three arms. The MPI varied from 0.0 to 13.3, 0.0 to 9.6 and 0.0 to 3.3 in the Arms 1, 2 and 3, respectively.

During 2012 and 2013, room spray coverage ranged from 22.8% to 51.8% and 46.8% to 60.4%, respectively. In 2014, the first round spray coverage was 50.7% (range: 37.5%–77.6%) in Laxmipur CHC and 42.0% (range: 32.7%–51.3%) in Dasmantpur CHC.

The project fund was sanctioned and the first instalment of the first year fund was received in November 2014. However, collection of base-line information as detailed above has been continued in the study areas from July 2012 with intramural budget.

1.3.4 Entomological and Epidemiological investigations on Leishmaniasis among the Kani forest Tribes in the tribal settlements of Thiruvananthapuram dt. Kerala

EM 1206: Mar 2012 – Feb 2015

Srinivasan R, Sabesan S, Pradeep Kumar N, Paily KP and Jambulingam P

Collaborating Institutes: *Directorate of Health Services, Government of Kerala (Dilip Kumar); Government Medical College, Thiruvananthapuram (Anish TS); Directorate of Animal Husbandry, Government of Kerala (Nandakumar S)*

In the southernmost part of the Western Ghats, known as Agasthyar Koodam, the Kani tribes inhabit in many settlements. These settlements located in the difficult-to-reach area were under the control of Kuttichal Primary Health Centre, Nedumangadu taluk, Thiruvananthapuram district, Kerala. The VCRC has investigated the prevalence of sandflies and occurrence of cutaneous leishmaniasis infection among the Kani tribes. Ecological conditions viz., abundant vegetation, heavy rainfall, warm temperature, high humidity and mud walled huts with loose and moist soil (**Figure 1.18**) favour sandfly abundance in these settlements. There has been no intervention measure implemented in the Kani tribe areas, due to paucity of information on seasonal abundance and distribution of sandfly vectors and behaviour. To design an intervention measure, extensive investigations on the entomological and epidemiological aspects of cutaneous leishmaniasis were carried out by the VCRC in the tribal settlements.

Objectives:

- To assess the prevalence and incidence of CL infection among the Kani tribes
- To incriminate vector species involved in transmission of CL
- To study the seasonal abundance and feeding behaviour of sand flies
- To assess the infection among animal reservoirs.
- To assess influence of climatic factors on sandfly abundance

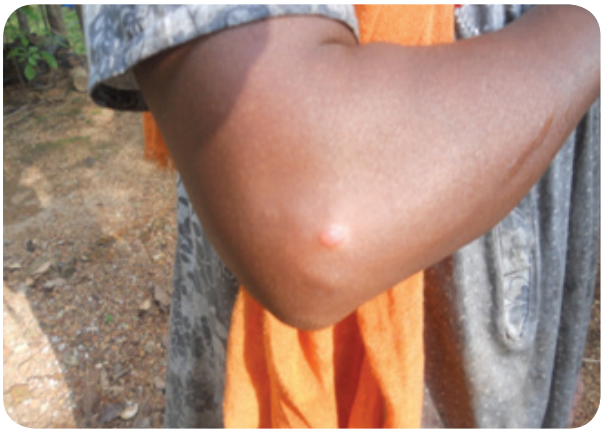
To know the extent of cutaneous leishmaniasis among the Kani tribes, door-to-door visits were completed and a total of 15 new / suspected cases either with nodule (**Figure 1.19**) or lesion were recorded. Skin/tissue samples were collected from cutaneous lesions of 13 of the 15 cases and subjected to histopathological examination at the Pathology Department, Government Medical College, Thiruvananthapuram, Kerala. Part of the tissue samples collected from all the 13 tribes were subjected to PCR based analysis for detection and identification of the parasite species.

LD bodies were found from skin/tissue samples of 3 of the 13 patients in histopathological examination. Among the 13 samples processed by PCR, 5 were found positive for *Leishmania* infection based on nested PCR amplification of kinetoplast mini-circle DNA. On analysis of these sequences, it was found that all the 5 samples belonged to *Leishmania donovani* with a cytosine molecule in the 634 region of hsp-70 gene. The sequences were submitted to GenBank and the accession numbers were obtained.

Investigation on the distribution and seasonal abundance of Phlebotomine sandflies were completed in 10 tribal settlements (criteria used for selection of on study area was given in previous annual report). During the reporting period, a total of 3942 sandfly specimens (males 18.3%, females 81.7%) comprising 16 species were obtained. (list of species recorded from the tribal settlements were given in previous report). Of the total specimens 86.8% were obtained from hand catch method, 9.3% from modified CDC light trap and 3.9%

FIGURE 1.18 Kani tribe dwelling

FIGURE 1.19 A CL cases with nodule



from sticky trap collections. Among the species obtained, *Phlebotomus argentipes* was the second predominant species (29.4%), next to *Sergentomyia baghdadis* (41.2%), indoors. Density of *P. argentipes* was the maximum during October 2013 (11.1 females/ PHR) and minimum in March 2014 (2.8 females/ PMR), among indoors in tribal dwellings.

Sandfly females (n = 662) collected from the tribal settlements were subjected for PCR assays. One pool of *P. argentipes* tested positive for natural infection with *Leishmania donovani* and the same was confirmed through sequencing.

A new sandfly species recorded from the tribal settlements was described and published. The species has been assigned a new name *Sergentomyia* (*Neophlebotomus*) *monticola sp. nov.* in recognition of the Western Ghats montane rain forests. The DNA barcode sequences of specimens collected from the study area, when analyzed showed that the overall genetic distance (K2P) between the sequences being only 0.4%, thus belonging to a single taxonomic category. Voucher specimens, comprising both holotype ♀ and allotype ♂ were deposited at the VCRC Museum. Besides, each paratype ♂ and ♀ was also deposited in the Smithsonian National Museum of Natural History (NMNH), Washington, D.C., USA.

Susceptibility of sandflies of the tribal areas was assessed to DDT and deltamethrin, using standard WHO testing procedure. A total of 720 sandflies comprising *P. argentipes* (70.6%), *S. bagdhadis* (14.4%), *Sergentomyia zeylonica* (8.2%), *Phlebotomus sintoni* (3.6%) and *Sergentomyia babu* (3.2%) were exposed for susceptibility. The number of *P. argentipes* females exposed to DDT (4%) and deltamethrin (0.05%) impregnated papers were 158 and 172 respectively, as this species was abundant in the total collection. Sandfly females (n = 120) were also exposed to respective control papers. *P. argentipes* and other sandfly species were found to be susceptibility to both DDT and deltamethrin.

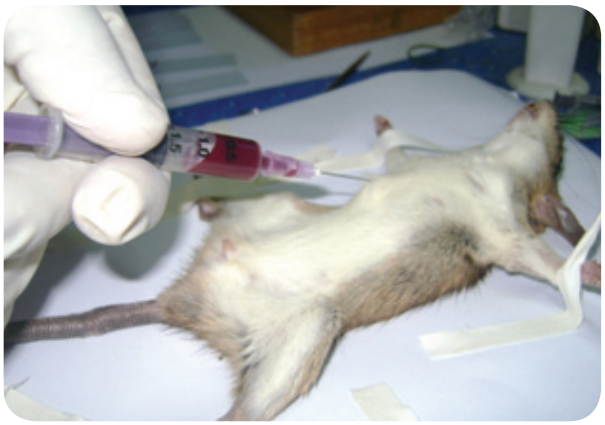
The tribes domesticate dogs to protect themselves from untoward activity of wild animals. Investigations on blood meal source of wild caught sandflies indicated that 48% (n = 160) of the sandfly females were found to have engorged blood meal either on double or triple hosts, during a single feeding. This finding of multiple hosts feeding of sandflies including human, dog and rodent, signifies the risk of involvement of animal reservoirs in CL transmission. Hence, domestic dogs and rodents found in tribal settlements were screened for CL infection. A total of 47 blood samples were collected and subjected for PCR assay and microscopy examination (**Figure 1.20**). The PCR test revealed that 3 blood samples were positive for *Leishmania* parasites. Further, the infection was confirmed through sequencing. When the blood smears collected from the dogs examined, LD bodies were seen in one of the dog blood samples, which was also positive for PCR assay.

Rodent surveys were also carried out simultaneously and a total of 25 rats, comprising two species viz., *Rattus rattus* and *R. norvegicus* were collected. From each rat, 2 ml blood, liver and spleen samples were collected (**Figure 1.21**). Tissue smears (both spleen and liver) were made to detect LD bodies, if any. All the samples were tested through PCR assay and none was found positive for CL infection. Blood smears and tissue smear (Liver and spleen) when examined microscopically, all were found negative for infection.

One of the priorities for control/prevention of CL is to create awareness among the community and ensure optimal utilization of interventions. First step in this direction was to carry out a situation analysis on prevailing Knowledge, Attitude and Practice (KAP) of inhabitants. A study among 103 respondents from 10 Kani tribal settlements showed that though 39.8% of respondents recognized pictures of CL shown to them, but did not have any lay perceptions. There was absolutely no awareness on vector, transmission, risk factors and control measures. The role of sandflies in CL causation was not known to the residents and this prevented them from using any personal protection and adhering to control measures which in turn pose risk of spread of infection within settlements and to newer areas. CL has not been recognized as a major disease and no treatment is taken for CL if symptoms do not persist. In the case of persons with ulceration and severe itching, use of natural herbs was reported by 90.2% (n = 37) among those who have recognized the disease (n = 41). Distance from health facility, difficult-to-reach nature of settlements in interior forest and lack of regular transport contribute to lack of accessibility to health information among inhabitants. It is essential to organize daytime training workshops and build capacity among them who in turn can deliver CL related health information to the rest of the tribal population and reinforce at frequent intervals with technical support from research group. This is expected to enable inhabitants to perceive CL as a health problem, thereby participating in control/elimination of CL.

FIGURE 1.20 Blood sample collection from dog

FIGURE 1.21 Blood sample collection from rat



1.3.5 Scrub Typhus: Establishment of disease and vector surveillance to assess the extent of disease occurrence and vector prevalence Project period: 3 years (2011–2014)

IM 1204: Oct 2011 – Nov 2014

Jambulingam P, Hoti SL, Sadanandane C, Patricia Anitha K (VCRC), Shashikala N, Reba Kanungo (PIMS, Puducherry)

Scrub typhus, caused by *Orientia tsutsugamushi* is the most prevalent Rickettsial infection in India and being increasingly reported from many parts of the country. It is an acute febrile illness transmitted by the larvae of *Leptotrombidium deliense*. Humans are accidentally infected. The case fatality can be significantly high, if the disease is not diagnosed on time and appropriately treated.

In the recent past, there has been a series of outbreaks of Scrub Typhus reported from various parts of the country indicating re-emergence of this disease. Incidence of scrub typhus cases have also been reported in and around Puducherry since 2006. The Puducherry has become now endemic for scrub typhus reporting cases every year. This study was undertaken to establish facilities and to build capacity for laboratory diagnosis of scrub typhus and to develop expertise on vector surveillance and identification at VCRC.

Objectives:

- To establish laboratory facilities for disease and vector surveillance through networking of medical institutions and hospitals in Puducherry and surrounding areas.
- To understand the immunological correlates to disease severity (immune pathogenesis) with reference to serotype/genotypes.

The epidemiological and clinical profiles of 145 clinically suspected scrub typhus cases were obtained from Pondicherry Institute of Medical Sciences, Indira Gandhi Medical College & Hospital, Aarupadai Veedu Medical College and Sri Manakula Vinayagar Medical College, Puducherry during 2011-2013.

Analysis of data showed that the cases were mainly from the coastal regions of Puducherry and nearby villages and from surrounding Tamil Nadu areas. Incidence of scrub typhus cases gradually increased from the mildly hot months of August and September and peaked in the cooler months (December and January). The age group analysis showed among the positives 67.5% were adults and 34.4% were of the paediatric age-group. Analysis of clinical profile showed that majority of the patients had only an acute febrile illness with no other complications, a few cases also manifested with meningitis and multi-organ disorder.

Blood serum samples were obtained from these clinically suspected cases (n = 145) and subjected to immunological and PCR diagnostic assays. Among the two immunological tests the IgM ELISA tested highest number of samples positive, almost twice as much as the conventional Weil-Felix test. Of the three PCR assays tested, the one based on detection of GroEL gene yielded significantly higher number of positives. All the three molecular markers (56 Kda antigen, 16s rDNA and GroEL genes) have been confirmed as that of *Orientia tsutsugamushi* by nucleic acid sequencing.

Determination of genotypes was done on positive samples in 56 kDA PCR, by Nucleic Acid Sequencing. A total of 6 genotypes were identified and most of the samples belonged to genotype ISS–11. Phylogenetic analysis of the genotypes have shown that some sequences are more similar to the Madhya Pradesh genotype and a few sequences are of genotypes unique to Puducherry.

Surveillance of *Leptotrombidium* mites (Chiggers), the vector of scrub typhus, was carried out in and around Puducherry district, where confirmed human cases of scrub typhus were reported. Mites were collected directly from the rodents/shrews trapped by Sherman live traps (7.6 × 8.9 × 22.9 cm) baited with peanut butter placed between 2 saltine crackers. Trap collections were made in 11 villages of Puducherry from October 2013 to September 2014. All the study locations were characterized by the presence of shrubs and bushes. The traps were set in peri-domestic areas of the selected villages an hour prior to sunset and retrieved the next day morning.

During the study period, a total of 134 rodents/shrews were trapped using 1067 Sherman traps, set in the 11 study villages. Overall, the trap positivity rate was 12.9%. Of the total 134 rodents trapped, *Rattus rattus* was the predominant species (48.5%) followed by *Suncus murinus* (39.6%), *Bandicota bengalensis* (11.2) and *Tatera indica* (0.7%).

A total of 6378 mites belonging to 9 species of trombiculids were recovered from the trapped rodents (**Table 1.8**). *Leptotrombidium (L) deliense*, the established vector of scrub typhus in India, was the predominant species (55.9%) followed by *Leptotrombidium insigne* (28.9%) and *Schoengastilla sp.* (7.8%).

Overall, the number of mites (all species of trombiculid mites) infested per rodent was 47.6. The number infested

TABLE 1.8 Species diversity of Trombiculid mites collected from the study villages in Puducherry

S.No.	Species	Number collected	Percentage
1	*Leptotrombidium deliense*	3564	55.9
2	*Leptotrombidium insigne*	1842	28.9
3	*Schoengastiella sp.*	499	7.8
4	*Trombicula hypodermata*	176	2.8
5	*Microtrombicula sp.*	114	1.8
6	*Schoengastia sp.*	63	1.0
7	*Helenicula sp.*	82	1.3
8	*Walchia sp.*	29	0.5
9	*Schoutedenichia sp.*	1	0.02
	Total	**6378**	

per rodent was higher with *Suncus murinus* (64.6) followed by *Rattus rattus* (42.2), *Bandicota bengalensis* (13.5) and *Tatera indica* (11.0) (**Table 1.9**).

The Chigger *(L. deliense)* index ranged from 11.0% to 88.5% in the villages surveyed and the overall Chigger index was 26.6%. In all villages surveyed, the Chigger *(L. deliense)* index was well above the critical level of chigger load i.e. 0.69 per rodent indicating that the villages are at high risk for outbreaks of scrub typhus.

Scrub typhus continues to cause an acute febrile illness in this region as evidenced both by the antibody detection and the detection of the DNA of the bacterium. The study demonstrated the prevalence and abundance of the chigger mite, *Leptotrombidium (L) deliense*, the known vector of scrub typhus, for the first time in Puducherry region. The higher infestation rates of chigger mite vectors observed in the present study is an evidence for the risk of transmission of scrub typhus in Puducherry.

TABLE 1.9 Infestation of mites per rodent (Chigger index)

Rodent/shrew species	Number of rats collected	Number of mites collected	No. of mites per rodent	No. of *L. delinse* collected	No. of *L. delinse* per rodent
Bandicota bengalensis	15	203	13.5	104	6.9
Rattus rattus	65	2742	42.2	1588	24.4
Suncus murinus	53	3422	64.6	1862	35.1
Tatera indica	1	11	11.0	5	5.0
Total	**134**	**6378**	**47.6**	**3559**	**26.6**

1.4.1 Studies on the transmission dynamics and control of Dengue in a forest fringe area of Kerala

IM 1303: Sep 2013 – Aug 2016

Pradeep Kumar N, Vijaya Kumar KN, Ambili Kumar, Snehalatha KS, Abidha, Krishnamoorthy K & Jambulingam P

Dengue is an emerging disease in India with rapid geographical spread. During 2013, as many as 75808 dengue cases with 193 deaths were reported in India. Kerala recorded the maximum number of cases (7938). All the four serotypes of dengue virus (DENV) have been detected in the State, including the first report of serotype 4 from this region (Kumar *et al.*, 2013). With an area of only about 1.5% of the country and about 2.8% of the population, Kerala state reported more than 13.1% of dengue cases in India for the last seven years. Cases were reported from all the fourteen districts. About 55% of the cases reported for the last seven years were from Thiruvananathapuram District.

The first case of Dengue in Kerala state was reported from Kanjirappalli taluk, Kottayam District during 1997 and is considered as its epicenter. This taluk is bordered by Western Ghats on its east and has vast forest fringe area which recorded about 82.0 % of dengue cases reported from this taluk. Forest fringe areas are considered as the gateway for the spread of zoonotic infections as Dengue to rural and urban areas. Maintenance of sylvatic cycle of DENV had been already reported from African and Malaysian regions (Wang *et al.*, 2000). Incidence of Dengue in Kottayam district commences with cases reported from forest fringe areas of Kanjirappalli town soon after the intermittent rainfall which occurs during summer season every year in Kerala state. In view of this, the present study is undertaken to assess the epidemiological and entomological risk factors which contribute for the high incidence in forest fringe areas and the possible zoonotic role in the transmission of dengue. Based on situation analysis, it is proposed to develop and demonstrate an IVM strategy, specific to the risk factors to control/prevent dengue outbreaks. Also this would provide information on the possible sylvatic cycle if any and its introduction to forest fringe areas and to the adjacent urban area, Kanjirappalli town.

Two villages in the forest fringe area and two wards in Kanjirapalli urban area have been selected based on the incidence of dengue cases (**Figure 1.22**). The demographic status of these four study sites and the incidence are given in the **Table 1.10**.

FIGURE 1.22 **Study Areas (highlighted regions are the study villages located on the forest fringes)**

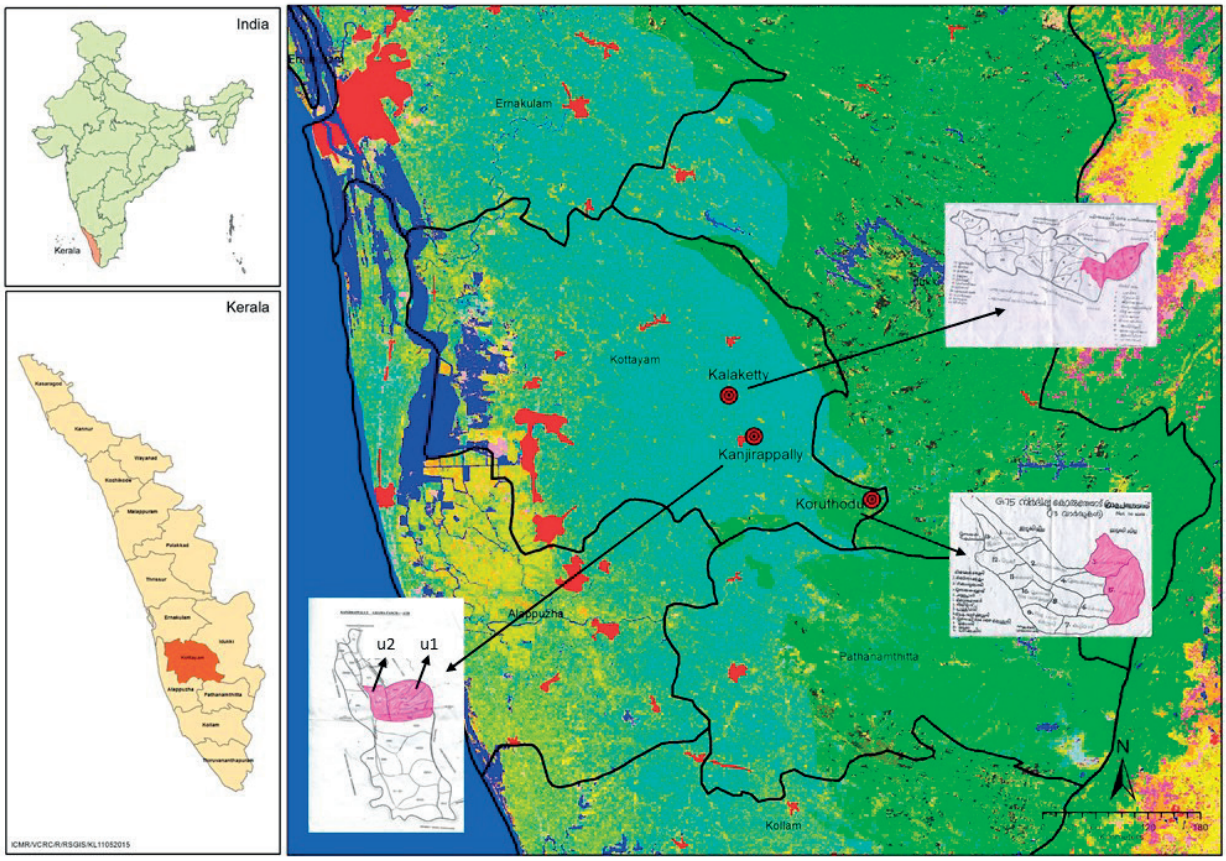


TABLE 1.10 Demographic status of the study sites and incidence

Study villages	Population	No. of households	Area (Sq. Km)
Kalaketti (FF1)	2592	525	2.5
Koruthode (FF-2)	2967	772	3.5
Kanjirappalli (U1)	2252	510	2.4
Kanjirappalli (U2)	1829	393	2.3

Objectives:

- To study relative abundance of Aedine vectors and to delineate their breeding habitats in the two settings.
- To conduct blood meal analysis of the wild caught mosquitoes to identify the source of blood meal.
- To monitor viral activity in vector population (both adult and immatures.
- To assess sero-prevalence of DENV in human population for estimating the magnitude of dengue transmission.
- To develop and demonstrate IVM strategy to prevent/contain of dengue outbreaks in these ecosystems.

Entomological surveys were carried at fortnightly intervals in the 4 areas. Both immature and adult collections were carried out following the standard procedures. Indoor resting collections were carried out using mechanical aspirators and spending 3 man hours in each area. Sampling of outdoor resting vector population was done by using sweep nets and spending 3 man hours. All the breeding sources in and around 12 households were enlisted and checked for vector breeding in an area of about 0.5 Sq Km. Samples of pupae and larvae collected from the positive sites were brought to the laboratory for emergence and species identification. Adult vector mosquitoes collected and adults emerged from pupal/larval samples were screened for dengue infection using RT-PCR.

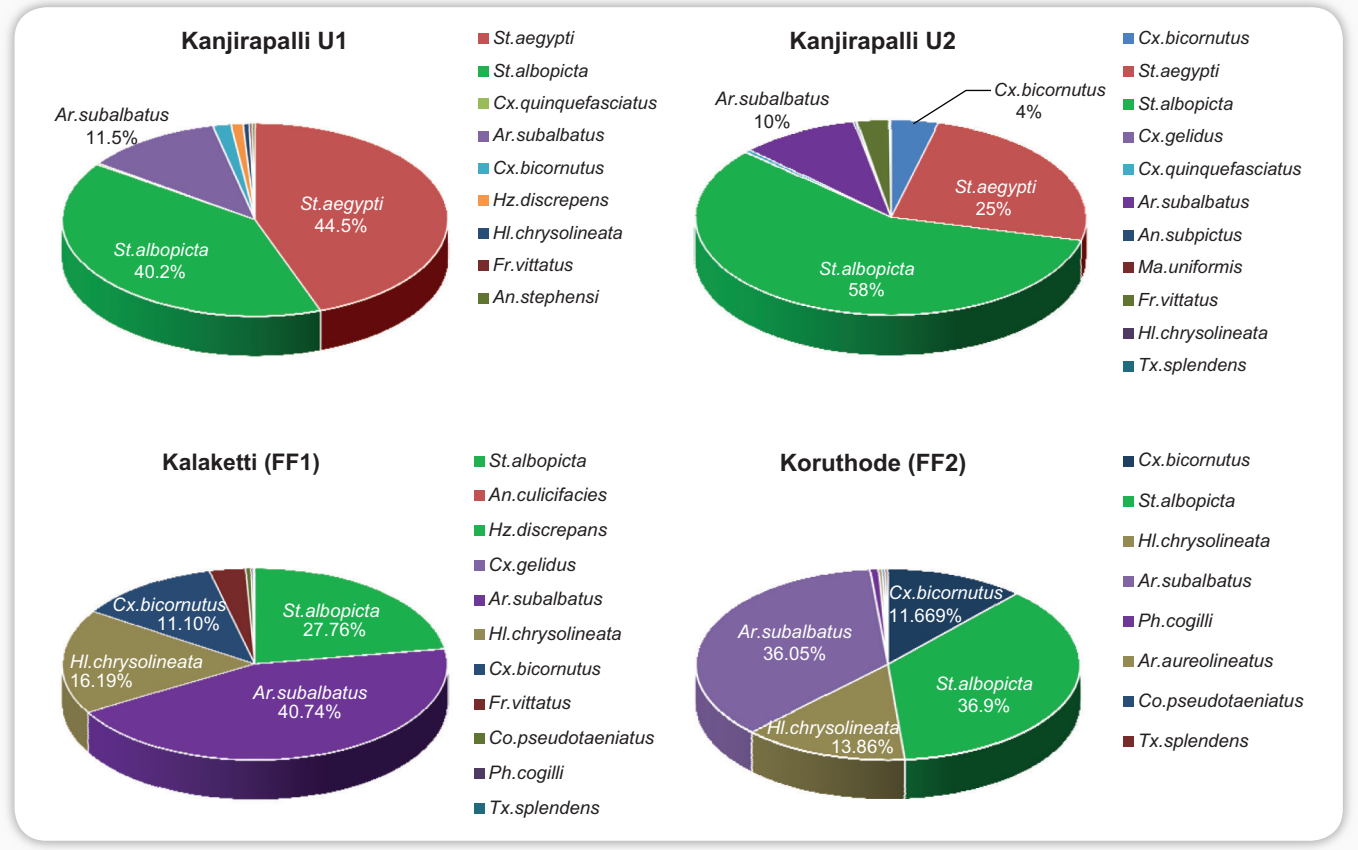
A total of 17 species of mosquitoes were recorded in the study area (**Table 1.11**). Six species belong to the Aedeine group have been recorded. Both *Stegomia aegypti* and *Stegomia albopicta* the implicated vectors of DENV were found to be abundant in Kanjrappalli town. However, *St. aegypti* was recorded only in the urban area. *St. albopicta* was the predominant species in all four areas (**Figure 1.23**).

Water storage containers were found to be the key breeding source for *St. aegypti*. Consequent to acute water storage in the urban area, storing water have become inevitable for

TABLE 1.11 Species composition (percentage prevalence) of mosquitoes in study areas

S.No.	Species	Kalaketty (FF1)	Koruthode (FF2)	Kanjirapally ward 9 (U1)	Kanjirapally ward 10 (U2)
1	*Stegomyia aegypti*	0	0	44.48	24.84
2	*Stegomyia albopicta*	27.76	36.9	40.19	57.90
3	*Armigeres subalbatus*	40.74	36.05	11.54	9.70
4	*Culex bicornutus*	11.1	11.7	1.52	3.94
5	*Heizmannia discrepens*	0.18	0	1.01	0
6	*Hulecoeteomyia chrysolineata*	16.19	13.86	0.51	0.07
7	*Culex quinquefasciatus*	0	0	0.25	0.48
8	*Fredwardsius vittatus*	0.28	0	0.25	2.65
9	*Anopheles stephensi*	0	0	0.25	0
10	*Anopheles subpictus*	0	0	0	0.20
11	*Culex gelidus*	0.47	0	0	0.07
12	*Mansonia uniformis*	0	0	0	0.07
13	*Toxorhynchites splendens*	0.12	0.24	0	0.07
14	*Collessius pseudotaeniatus*	0.41	0.24	0	0
15	*Phagomyia cogilli*	0.18	0.72	0	0
16	*Anopheles culicifacies*	0.06	0	0	0
17	*Armigeres aureolineatus*	0.0	0.3	0	0
	Total	**1711**	**1667**	**1187**	**1473**

FIGURE 1.23 Species composition of mosquitoes in urban (a & b) and forest fringe (c & d) study areas



the community. Plastic drums, cement tanks and cisterns are used to store water in these areas. Peridomestic water storing containers accounted for 62.71% of the total 718 immatures collected from these sites. Peri-domestic discarded containers (40%) were the main breeding habitats of *St. albopicta* (n = 982) in urban areas. In forest fringe areas, 29.9% of breeding of *St. albopicta* (n = 897) was contributed by rubber plantation associated latex collection containers (either unused or discarded) followed by peri-domestic discarded containers (27.83%). Small scale rubber plantation is common and interspersed in the forest fringe areas. The area wise contribution of different habitats is provided in **Table 1.12**.

As many as 541 adult mosquitoes of and 1589 immature stages of *St. albopicta* were collected during October 2013 to

TABLE 1.12 Habitats enlisted for *St.albopicta* breeding in study areas

Type of Breeding habitats	Kanjirappalli U1		Kanjirappalli U2		Kalaketty	Koruthode
	St.aegypti	*St.albopicta*	*St.aegypti*	*St.albopicta*	*St.albopicta*	*St.albopicta*
I. Peridomestic						
1. Water storage containers	28 (70.0)	15 (39.5)	9 (47.4)	9 (19.2)	11 (20.4)	8 (18.6)
2. Discarded materials:						
• *Containers / utensils*	6 (15.0)	10 (26.3)	5 (26.3)	24 (51.1)	15 (27.8)	12 (27.9)
• *Tyres*	1 (2.5)	1(2.6)	1 (5.3)	4 (8.5)	8 (14.8)	3 (6.9)
3. Rubber plantation associated containers	0 (0.0)	9 (23.7)	0 (0.0)	7 (14.9)	12 (22.2)	17 (39.5)
4. Natural (Tree holes / plant axils) breeding habitats	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	3 (5.6)	1 (2.3)
II. Domestic (Fridge trays)	3 (12.5)	3 (7.9)	4 (21.1)	2 (4.3)	5 (9.3)	2 (4.7)

November 2014. This species was recorded throughout the year with a peak in pupal indices during February to June (**Figure 1.24**). A similar trend was observed in all the areas and the seasonality depends on the availability of breeding sources. The stegomyia indices through different months for all the four study areas is given in **Table 1.13**. Unused latex collection containers and water storage containers support profuse vector breeding. *St. aegypti*, found only in the urban area and was recorded during the summer season (**Table 1.12**) when the rainfall was minimal and people tend to store water. Temporal and spatial variations in vector population was observed which is useful in designing vector control strategies.

A total of 564 specimens of *St. aegypti* (279 females & 285 males) collected in adult/immature collections were processed from Kanjirappalli village, (U1 & U2) during the study period in 133 pools. The specimens were segregated according to the abdominal conditions (UF, FF, SG & G). Out of

FIGURE 1.24 Pupal index in different months in relation to Rainfall

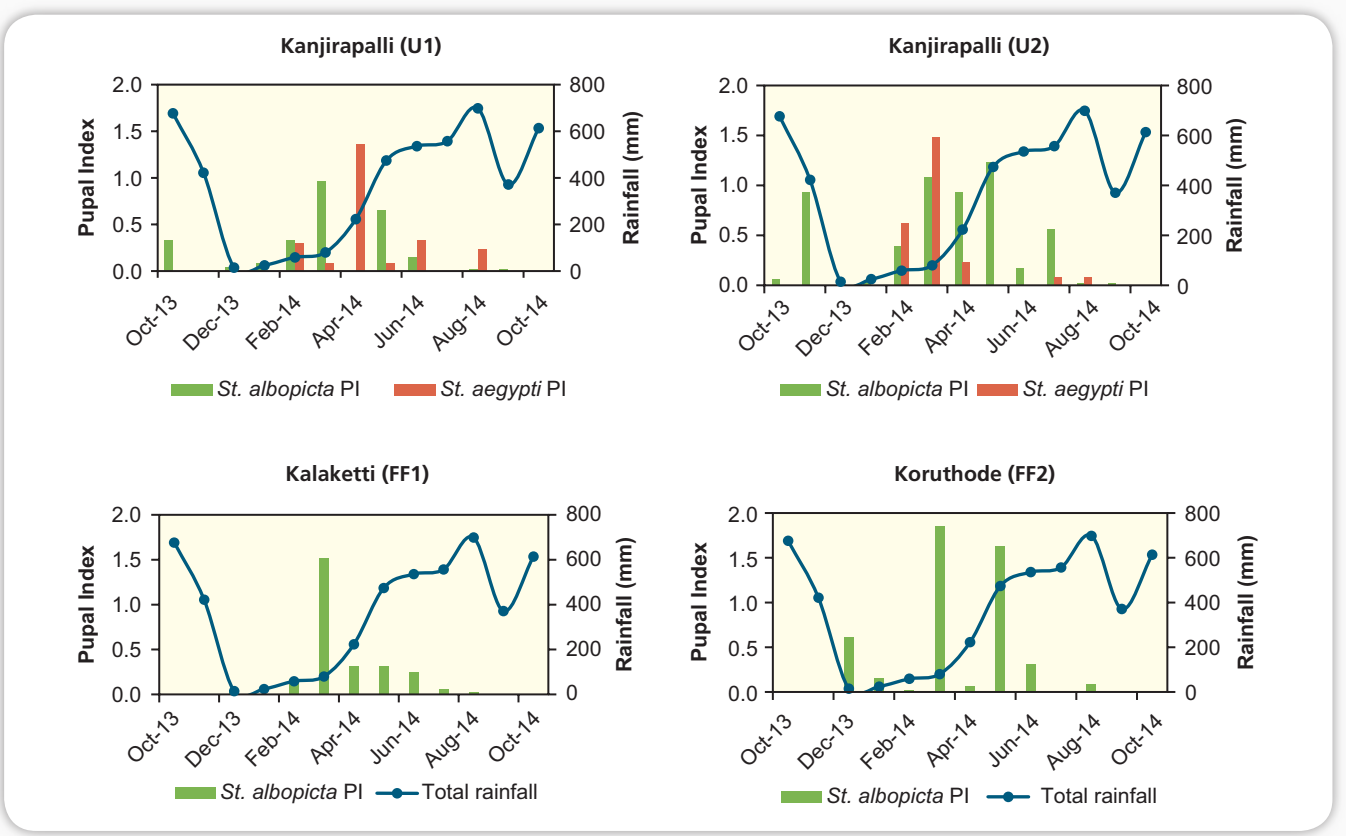


TABLE 1.13 Stegomyia breeding indices in different study areas

	Kanjirappalli U1						Kanjirappalli U2						Kalaketty			Koruthode		
	St. aegypti			*St. albopicta*			*St. aegypti*			*St. albopicta*			*St. albopicta*			*St. albopicta*		
Months	CI	BI	PI	CI	BI	PI	CI	BI	PI	CI	BI	PI	CI	BI	PI	CI	BI	PI
Oct-13	0.00	0	0.00	75.00	6.25	0.33	0.00	0	0.00	7.69	2.08	0.06	4.17	4.16	0.00	0.00	0.00	0.00
Nov-13	0.00	0	0.00	40.00	4.16	0.00	0.00	0	0.00	100	14.60	0.94	5.26	2.08	0.00	15.38	4.16	0.00
Dec-13	0.00	0	0.00	4.17	2.08	0.04	0.00	0	0.00	0.00	0.00	0.00	50.00	8.33	0.02	27.03	20.83	0.60
Jan-14	0.00	0	0.00	100	4.16	0.08	0.00	0	0.00	50.00	2.08	0.04	0.00	0.00	0.00	37.50	6.25	0.15
Feb-14	15.91	14.6	0.29	18.18	16.66	0.33	17.39	8.33	0.63	30.43	14.60	0.40	6.90	4.16	0.17	20.00	2.08	0.02
Mar-14	5.56	4.16	0.08	11.11	8.33	0.96	14.71	1.04	1.48	23.53	16.66	1.08	16.85	31.25	1.52	16.67	16.66	1.85
Apr-14	16.67	18.75	1.35	3.70	4.16	0.00	8.11	6.25	0.23	27.03	20.83	0.94	16.36	18.75	0.31	9.09	8.33	0.06
May-14	8.89	8.33	0.08	17.78	16.66	0.65	0.00	0	0.00	23.53	16.66	1.23	13.79	25.00	0.31	13.04	18.75	1.63
Jun-14	13.85	18.75	0.33	7.69	10.42	0.15	0.00	0	0.00	11.43	8.33	0.17	7.58	10.42	0.25	5.75	10.42	0.31
Jul-14	2.86	2.08	0.00	0.00	0	0.00	5.41	4.16	0.08	10.81	8.33	0.56	7.14	6.25	0.06	1.85	2.08	0.00
Aug-14	23.53	16.66	0.23	8.82	6.25	0.02	11.54	6.25	0.08	3.85	2.08	0.02	1.89	2.08	0.02	0.00	0.00	0.08
Sep-14	0.00	0	0.00	1.75	2.08	0.02	8.33	4.16	0.00	4.17	2.08	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Oct-14	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0.00	0.00	1.19	2.08	0.00	0.00	0.00	0.00

133 pools of *St. aegypti*, one pool of SG with 6 specimens and another pool of UF with one specimen from U1 were found positive for DENV infection. The positive samples were from indoor resting collections. While the pool with SG showed infection with DENV4 & DENV2 serotypes, UF specimen was found with DENV4 (**Table 1.14**). The genetic characterization of DENV4 recorded in *St. aegypti* showed close genetic relatedness to the DENV4 isolate from the human Dengue case recorded from Kanjirappalli during the study period. The results were communicated to the Dept. of Health services and as a rapid response, fogging was carried out to prevent transmission. *St. albopicta* specimens were also screened for arbo-viral infection. Processing of 195 pools with 756 specimens (473 females & 283 males), collected from all the four sites and none of the pools showed positive for DENV infection. Among these 222 specimens were collected as adults and 534 were from adult emergence of immature collections.

DNA barcode analysis was done to determine the source of blood meal of the mosquitoes and vector association with the animal and human hosts. The COI sequences of the vertebrate host-species of blood meal were amplified and are were custom sequenced. So far 18 specimens were subjected to this analysis and all of these were found to be from *Homo sapiens*.

Serum samples from the cases reported to hospitals in Kanjirappalli urban area with dengue symptoms from the study villages were also processed to detect and confirm infection by RT-PCR. Two out of 9 samples showed positive, one with DENV4 and another mixed infection with DEN1 & DENV4.

1.4.2 Demonstration of mosquito vector control and prevention of dengue/ chikungunya through partnership and community empowerment in selected rural areas of Puducherry

IM 1304: Jan 2013 – Dec 2016

Krishnamoorthy K and Nandha B

Collaborators: NVBDCP, Puducherry

Aedes transmitted dengue and chikungunya are being recorded in recent times from all the areas in Puducherry. First report of dengue was in 2003 and from 2011 dengue cases are reported every year and in 2012, as many as 3506 suspected with 5 deaths were reported. Sentinel surveillance centres confirmed 1191 cases. In 2013 and 2014 there were 2215 and 466 cases respectively. The problem has become

TABLE 1.14 Arbo-viral infection status of *Stegomyia* mosquitoes collected from forest fringe areas

Area	Specimens	Adult collections						Emergence					
		St. aegypti			*St. albopicta*			*St. aegypti*			*St. albopicta*		
		No. of mosquitoes processed	No. of Pools	No. of Pools +ve	No. of mosquitoes processed	No. of Pools	No. of Pools +ve	No. of mosquitoes processed	No. of Pools	No. of Pools +ve	No. of mosquitoes processed	No. of Pools	No. of Pools +ve
Kalaketty	Male	1	1	N	0	NA	NA	61	20	N	0	NA	NA
	Female	5	4	N	0	NA	NA	76	28	N	0	NA	NA
	Total	**6**	**5**	**N**	**0**	**NA**	**NA**	**137**	**48**	**N**	**0**	**NA**	**NA**
Koruthode	Male	2	1	N	0	NA	NA	42	12	N	0	NA	NA
	Female	10	4	N	0	NA	NA	61	16	N	0	NA	NA
	Total	**12**	**5**	**N**	**0**	**NA**	**NA**	**103**	**28**	**N**	**0**	**NA**	**NA**
Kanjirappally (U1)	Male	22	5	N	22	5	0	56	14	N	97	24	N
	Female	64	7	N	49	15	*2	61	17	N	96	27	N
	Total	**86**	**12**	**N**	**71**	**20**	**2**	**117**	**31**	**N**	**193**	**51**	**N**
Kanjirappally (U2)	Male	28	4	N	25	5	N	71	19	N	141	26	N
	Female	90	17	N	22	6	N	106	26	N	112	25	N
	Total	**118**	**21**	**N**	**47**	**11**	**N**	**177**	**45**	**N**	**253**	**51**	**N**

**1 pool from UF (1 specimen) – positive for DENV4 infection & 2nd pool from SG (6 specimens)-positive for DENV 2&4 infection.*
NA – Not Applicable. N – Negative

acute particularly in rural areas where there are no organized vector control activities. Chikungunya was recorded in 2006 with 542 clinical cases 9 of which were positive serologically. In view of increasing trend of vector borne diseases, this study aims at developing and demonstrating integrated vector management (IVM) focusing on fostering inter-sectoral collaboration and empowering communities for the prevention and control of dengue. Community Readiness assessment study also indicates low level community readiness in undertaking preventive measures, demanding a system for constant motivation.

Two PHCs viz., Manadipet and Ramanathapuram with population of 15331 (9 villages) were selected as intervention arm and one PHC (Thirukanur) with a population of 10528 in five villages as control arm. Dengue cases are reported every year in these PHCs. Base line surveys showed high environmental risk (79.9% of the households with vector breeding sources) with a mean number of 3 habitats per house. Vector breeding indices were also found to be high (container index 47.4%; breteau index 3.7% and house index 3.1%). Despite daily supply of water, practice of storing water in earthen pots and cisterns continues in all the villages. Water storing containers constituted about 75.2% of the total vector breeding sources enumerated. Schools, Self-help groups, NGOs, and Neighborhood committees have been identified as potential stakeholders in motivating the community and mobilizing their participation besides monitoring the risks of vector breeding (**Table 1.15**). Clearing of discarded containers/utensils, Discouraging the practice of discarding utensils/containers (rain dependent) and their clearance as well as keeping the water storage containers (cisterns and plastic drums) are the key messages besides demonstrating source reduction during the house visits are the task entrusted with the volunteers.

As many as 192 students (IX & XIth std) were trained through 12 orientation classes in four schools. These students in five batches made fortnightly visits and covered 431 households in 50 visits in the allocated study area. Students also made a script on dengue and community role in vector management and enacted street dramas. The mean number of discarded containers was brought down from 3.3 to 1.8 (45.3%) while the water storage containers remain unchanged (2.6 vs. 2.4). In the comparison area 300 and 754 houses visited by the research team during the corresponding pre and post intervention period showed that the mean number of discarded containers was 4.1 and 3.9 respectively and the water storage containers were 2.8 and 2.9 respectively. None of the 106 earthen pots, 7.5% (3/40) of the cisterns and 1.5% (5/321) of the drums were found positive for vector breeding in the area under school based approach. Independent assessment showed that the mean number of discarded containers per house was lower (3) in the houses visited by the students compared with houses (5.2) yet to be visited.

23 members from four SHGs, 11 members from an NGO (MS Swaminathan Research Foundation) and 183 members from 9 neighbourhood committees formed in two villages were trained and identified as community volunteers to motivate the community besides monitoring vector breeding in the allocated villages. As many as 79, 249 and 224 houses were visited by the members of SHGs, neighbourhood committees and NGO respectively in their allocated villages. A reduction in the mean number of containers was observed in the villages adopted for NGO was observed while in the other villages it remained at the level observed in comparison areas.

In the areas under school based approach, the mean number of discarded containers was lower (4) when compared to comparison area (8.2) during the post intervention period. Overall, there was a drastic reduction in the number of discarded containers (72% of the utensils and 25 % of the plastic containers) in the intervention areas, indicating that the discontinuation of practice of discarding containers by the community. Process and impact evaluation is in progress.

TABLE 1.15 Stakeholders involved in the community participation / mobilization

Approach	No. villages	Target population
School students	4	8245
SHG	1	1128
NGO	2	4201
Neighbourhood committee	2	1757

1.4.3 Forecasting JE mosquito vectors abundance through Geo Environmental risk determinants, using Remote Sensing & GIS

EM 1130: Mar 2011 – Feb 2014

Sabesan S (till Jan 2014), Rajavel AR, Raju KHK, Subramanian S, Natarajan R & Jambulingam P

The results of this study carried out till the end of December 2013 have been furnished in the earlier annual report.

Objectives:

- To ascertain the relationship between JE mosquito vector(s) abundance with different stages of paddy cultivation and other environmental variables.
- To link RS imageries and environmental variables corresponding to ground characteristics.
- To develop model forecasting JE vector abundance and transmission risk based on underlying relationship between environmental determinants and paddy cultivation, using Remote Sensing & GIS.

The study was carried out in JE endemic areas in two districts viz., Cuddalore in Tamil Nadu and Bellary in Karnataka. A total of three villages, one each from three different PHCs in each district were selected, where paddy cultivation is in practice. Vector abundance (immature density and adult density) and paddy growth stages were monitored for a period of 30 months. Satellite data (RISAT-1) corresponding to the different stages of paddy growth was

obtained from National Remote Sensing Centre, Hyderabad, using which the backscatter coefficient (σ^0) was derived.

The abundance of JE vector, *Culex tritaeniorhynchus* peaked when the paddy was at its heading stage and dipped when the crop reached the maturing stage. A significant correlation was observed between paddy growth and adult vector density in the study sites (Bellary: $r = 0.73$, $P < 0.008$; Cuddalore: $r = 0.77$, $P < 0.003$).

The antigen capture ELISA tests revealed the presence of JE virus in *Cx. tritaeniorhynchus* in 'Rabi' as well as 'Kharif' seasons. The minimum infection rate (MIR / 1000) ranged from 0.0 to 14.18 in Bellary and 0.0 to 14.5 in Cuddalore. Relatively a higher MIR was observed during hotter months when the vector density was low.

The sigma naught (σ^0) values derived for Bellary ranged from -18.3 (during the transplantation stage) to ~ -10 (during non-cultivation (dry / summer) period. A significant positive correlation was observed between σ^0 and paddy growth stages ($r = 0.87$, $p < 0.05$), and adult vector density ($r = 0.74$, $P = 0.04$). The σ^0 value observed during the vegetative and flowering stages of paddy growth ranged from -17.6 to -17.16 at which period the vector density started building up and hence this could be the spectral signature which denotes the 'risk' following which a high vector abundance is expected.

It was possible to identify the 'risk' specifically for the areas where the conventional paddy cultivation is in practice (Bellary district). We need to work out a different strategy for areas where there is mixed cultivation (Cuddalore district).

1.4.4 Research-cum-intervention project on JE/AES - Vector control to minimize the risk of transmission of JE in Gorakhpur District

EM 1208: 2013 – 2015

P. Jambulingam, A. R. Rajavel, S. Subramanian and K. Gunasekaran

Eastern Uttar Pradesh especially Gorakhpur and Basti Division have been facing the problem of seasonal encephalitis for about 35 years. Uttar Pradesh contributes about 60% of the total encephalitis burden of the country. Besides the Japanese Encephalitis (JE) virus transmitted by the mosquito, other water borne etiological agents such as the enterovirus have been found to be responsible for this high burden of Acute Encephalitis Syndrome (AES). The Expert Group Meeting to assess the situation of JE/AES in the country recommended that ICMR should undertake a research-cum-intervention project to (1) study the vector dynamics, circulation of viruses in vectors, transmission in pigs/other animals, (2) assess the impact of vector control method and other interventions. In the subsequent meetings about planning the research-cum-intervention project, it was decided that this will be a multi-institutional project carried out by ICMR institutes namely, the National Institute of Epidemiology, National Institute of Virology, Enterovirus Research Centre, Vector Control Research Centre and Centre for Research in Medical Entomology.

Objectives:

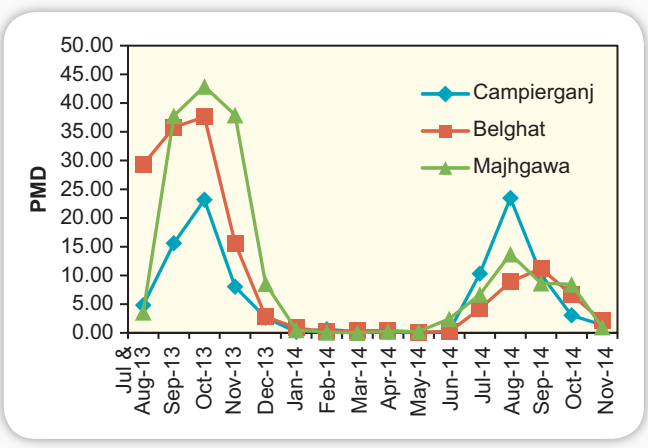
- To generate detailed information on the bionomics of the vector for extended intervention plan.
- To plan and implement measures for reducing man-vector contact at block level for JE prevention/control

Regular surveys for collection of entomological data were continued in all the 15 study villages. Fortnightly collections were made in the villages Bahadurpur, Bharsi, Barigaon, Harpur and Gayghat of Belghat intervention block, villages Sarpatha, Machligaon, Ramnagar, Shivpur and Kaharpurwa of Campierganj intervention block of District Gorakhpur and in Katrari, Pidara, Lahilpar khas, Babhani and Saraura in the comparison Block Majhgawa of District Deoria. Indoor resting collections were done in fixed catching stations of human dwellings, mixed dwelllings and cattle sheds and in outdoor habitat in each of the study village for determining the per man hour density. Unfed females were pooled for determining the Minimum Infection Rate (MIR) and stored in -80°C. Blood meal samples were taken on filter paper from fully engorged females for determining Human Blood Index (BMI). Both the samples were transported to the laboratory at VCRC for processing and analysis.

The per man hour resting density of the vector *Culex tritaeniorhynchus* in the intervention blocks Campierganj & Belghat and in the comparison block Majhgawa is given in **Figure 1.25**.

The per man-hour density of the JE vector Culex tritaeniorhynchus showed an increase from July/August 2013, reaching peak density of 23.17, 37.6 and 42.9 per man hour respectively in Campierganj, Belghat and Majhgawa blocks in the month of October 2013. Following this, there was a sharp decline in December 2013 and the density remained low up to May 2014, with density going down to less than 1 per man-hour. The density began to increase again from the month of July in 2014. It was high in August 2014 with 12.8, 23.46 and 8.89 per man in Campierganj, Belghat and Majhgawa respectively (much below than that of last Year). The density declined in the months of September, October and November 2014 and was much lower than that recorded during the same months of 2013. This was due to the deficient monsoon of the current year (**Figure 1.25**).

FIGURE 1.25 **Per man-hour density of *Culex tritaeniorhynchus* in Blocks Campeirganj, Belghat and Majhgawa (July–August 2013 to November 2014)**



The indoor density of *Cx. tritaeniorhynchus* was generally lesser than the outdoor density (**Figures 1.26, 1.27 & 1.28**). Between the indoor habitats, density in the human dwellings was comparatively lower than that in cattle sheds and mixed dwellings. Outdoor collections made in close proximity to the pigsties showed that the density of the vector was lesser than that recorded in other outdoor habitats except in Block Majhgawa during September 2014.

The density of the other two vector species, namely, *Cx. vishnui* and *Cx. pseudovishnui* was almost negligible in all the three blocks (**Figures 1.29, 1.30 & 1.31**).

FIGURE 1.26 **Indoor and Outdoor density of *Cx. tritaeniorhynchus* in Campeirganj Block (LLIN)**

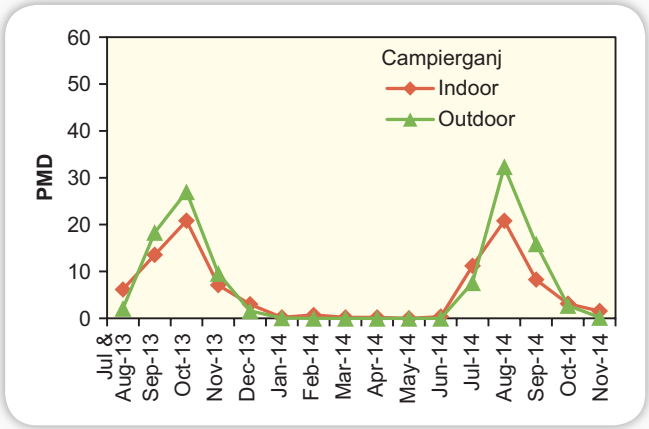


FIGURE 1.27 **Indoor and Outdoor density of *Cx. tritaeniorhynchus* in Belghat Block (IRS)**

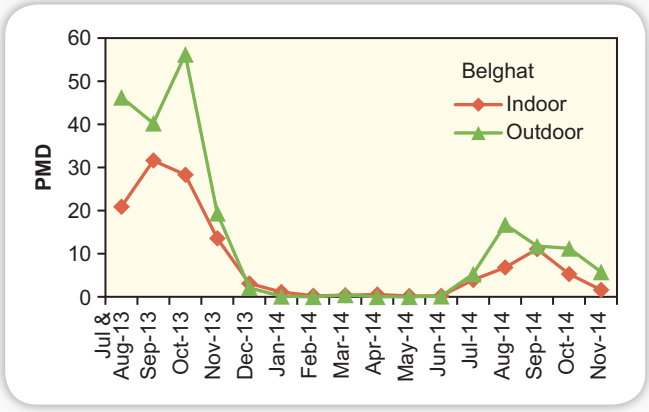


FIGURE 1.28 **Per-man-hour density of *Cx. vishnui* and *Cx. pseudovishnui* in Campierganj Block**

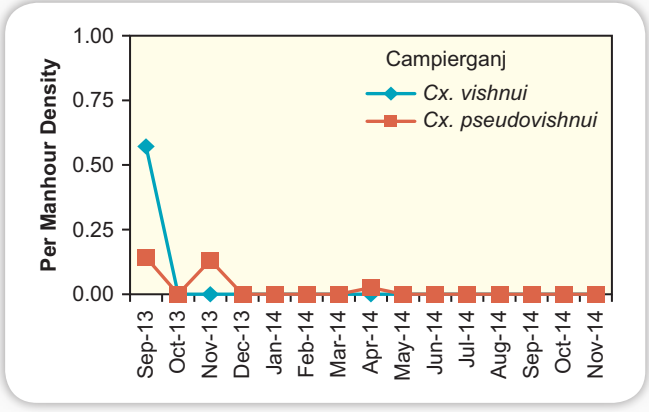


FIGURE 1.29 **Indoor and Outdoor density of *Cx. tritaeniorhynchus* in Majhgawa Block (Control)**

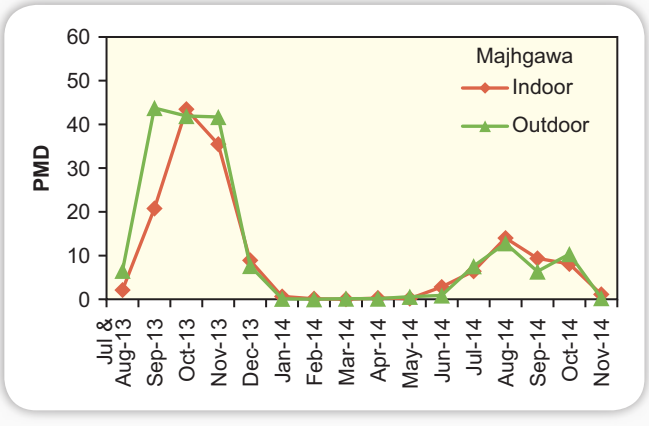


FIGURE 1.30 **Per-man-hour density of *Cx. vishnui* and *Cx. pseudovishnui* in Belghat Block**

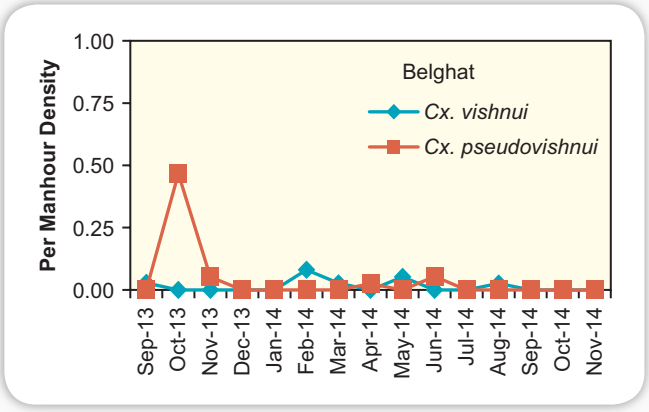
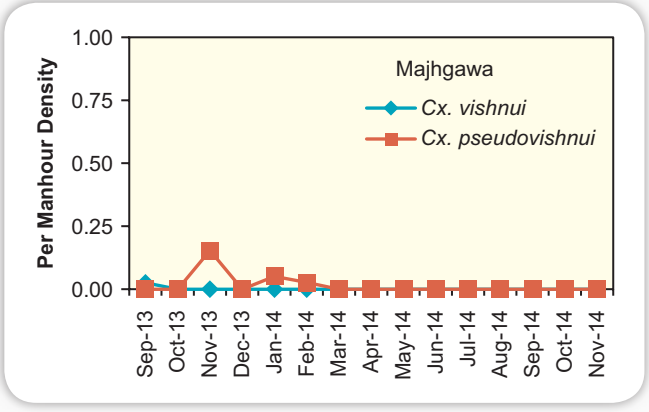


FIGURE 1.31 **Per-man-hour density of *Cx. vishnui* and *Cx. pseudovishnui* in Majhgawa Block**



A total of 1112 samples were collected and analyzed for blood meal identification. The BMI for humans, bovine and pig were respectively 0.005, 0.91 and 0.04 in Campierganj and 0.007, 0.93 and 0.01 in Belghat intervention blocks while it was 0.002, 0.97 and 0.002 respectively in the control block of Majhgawa. Blood meal analysis has shown that the JE vector *Cx. tritaeniorhynchus* predominantly feeds on cattle.

For JE virus detection in mosquitoes, a total of 3480 mosquitoes (in 176 pools) from Campierganj and Belghat intervention blocks of Gorakhpur district and Majhgawa control block in Deoria were collected and analyzed. A total of 8 pools in the intervention blocks and 7 pools in the control block were positive for JEV. MIR for intervention and control blocks was 4.59 and 4.03 respectively.

Basic outline of the intervention strategy is as follows: Indoor Residual Spray (IRS) with Lambdacyhalothrin 10% WP will be carried out in 2 Blocks, namely, Belghat and Bhathat. Long Lasting Insecticidal Net (LLIN) will be distributed in 1 Block, namely, Campierganj. Majhgawa block of the Deoria district will remain as the control for comparison.

In view of the delay in the procurement of the materials (LLIN, Insecticide and sprayers) and low vector density during 2014 due to deficit monsoon rains, the interventions will be implemented in June-July 2015 to interrupt the buildup of vector density. Implementation plan is being prepared in consultation with the State/District Health Department.

1.4.5 Preliminary survey of Kyasanur Forest Disease virus in ticks and antibodies in rodents in potential risk areas of adjoining States to Karnataka (Collaborative project with NIV)

IM 1407: Apr 2014 – Mar 2016

C Sadanandane, HK Raju K and A Elango.
Project coordinators: P Jambulingam, Director, VCRC and DT Mourya, Director, NIV

Kyasanur forest disease (KFD) was first reported from Kyasanur forest in Shimoga district of Karnataka in 1957 and until 1971 it was confined to three taluks of the district. Thereafter, it has been reported from four other additional foci (Uttar Kanara, Dakshina Kannada, Chikkamagalore and Udipi districts) of Karnataka State. In 2012, an outbreak of KFD was reported in Chamarajanagar district of the State for the first time. Later in 2013, the KFD virus was detected in autopsy of dead monkeys in Nilgris district, Tamil Nadu and in a human case from Wayanad district, Kerala. These reports indicate that the disease is spreading to the other districts of Karnataka and also adjoining States. Therefore, there is an urgent need to assess the risk by monitoring/screening ticks and rodent reservoirs for KFD virus in the forest fringe areas of Karnataka and adjoining states and the emerging potential of Kyasanur forest disease in the newer areas.

Objectives:

- To investigate the presence of KFD virus in ticks and antibodies in rodents from potential risk areas of Karnataka and adjoining States.
- To determine the environmental risk factors that favours the circulation of KFD virus in the potential risk areas.

An endemic area (Shimoga district, Karnataka) and four potential risk areas (Chamarajanagar district (Karnataka), Wayanad district (Kerala), Nilgiris and Erode districts (Tamil Nadu) were selected for the study based on the recent incidence/report of KFD cases (Map). In each district, forest fringe localities that are adjacent to the areas with recent incidence of KFD virus in humans / monkeys were chosen.

The project proposal was approved by the SAC (2013) and submitted to ICMR Task force for KFD and funding is awaited. In the meanwhile, a preliminary survey was carried out jointly by VCRC and NIV team in Shimoga, Chamrajnagar districts of Karnataka, Wayanad district of Kerala and Nilgiri district of Tamil Nadu during 01-04-2014 to 10-04-2014 for screening of KFD virus in ticks and rodents.

Collection of ticks and rodents was carried out in two villages of Thirthahalli Taluk, Shimoga district, Karnataka, where an outbreak of KFD was reported in 2014, one village in Maddur division of Bandipur, Chamarajanagar district, Karnataka, where KFD virus in both humans and monkeys was detected in 2012, one site at Muthanga forest range, Wayanad district, Kerala, where KFD virus in human was detected in 2013 and one site at Abayaranyam forest range, Mudumalai, Nilgiri district, Tamil Nadu, where KFD virus was detected from autopsy of dead monkeys in 2013 (**Figure 1.32**).

FIGURE 1.32 Map showing study sites at Shimoga, Chamaraj nagar, Wayanad, and Nilgiri districts

In each of the selected site, ticks from the forest floor were collected by flag dragging using lint clothes (100x70 cm). A total of 623 tick (nymphs) specimens belonging to 5 species of genus *Haemaphysalis* and one species of *Ambylomma* was collected. *Haemaphysalis spinigera*, the primary vector of KFD virus formed 83.7% of the total ticks collected. Other *Haemaphysalis* species collected were *H. turturis* (10.1%), *H. bispinosa* (2.7%), *H. intermediata* (0.5%) and *H. cupsidata* (0.2%). *Ambylomma* species formed 2.9% of the total ticks collected (**Table 1.16**).

The overall density of *Haemaphysalis spinigera* was 17.4 per man-hour in all areas surveyed. The densities of *H. turturis, H. bispionsa* and *Ambylomma sp.* were 2.1, 0.56 and 0.60 per man-hour, respectively. District-wise analysis showed that the density of *H. spinigera* was the highest in Alegowdankatte site, Chamarajnagar district (32.8) followed by Thirthahalli taluk, Shimoga (17.7), Abayaranayam, Nilgiri district (13.3) and Muthanga, Wayanad district (0.25) (**Table 1.17**).

In addition, trapping of rodents was done at Thirthahalli taluk, Shimoga district and Mulehole forest range, Bandipur division, Chamaraj Nagar district, Karnataka using sherman traps. In total, 240 traps were set in the selected forest fringes and the trap positivity was 5.4%. During the survey, 13 rodents belonging to 6 species viz., *Rattus golunda* - 5, *Rattus blanfordi* - 4; *Suncus murinus* - 1; *Mus saxicola* - 1; *Mus boodugu* - 1and *Mus platythrix* - 1 were trapped.

From the trapped rodents, blood serum and tissue (lung, liver and kidney) samples were collected. All the tick samples (pooled in 46 vials) and the serum and tissue samples (60 pools) of rodents were labelled with codes and sent to the NIV, Pune in liquid nitrogen containers for KFD virus identification and isolation.

The RT-PCR assay results showed that one pool (tick) sample from Thirthahalli taluk, Shimoga district and another from Mudumalai, Nilgiri district, Tamil Nadu were positive for KFD virus. Virus isolation by tissue culture is being carried out at NIV, Pune.

TABLE 1.16 Species diversity of ticks (nymphal stage) collected from Shimoga, Chamaraj nagar, Wayanad and Nilgri districts

S.No.	Species	No. Collected	Percentage
1	*Haemaphysalis spinigera*	522	83.7
2	*Haemaphysalis turturis*	63	10.1
3	*Haemaphysalis bispinosa*	17	2.7
4	*Haemaphysalis intermedia*	3	0.5
5	*Haemaphysalis cuspidata*	1	0.2
6	*Ambylomma sp*	18	2.9
	Total	**624**	

TABLE 1.17 Distribution of *Haemaphysalis* species in four districts

S.No.	Species	Shimoga (MHS: 13)	Chamaraj nagar (MHS: 6)	Wayanad (MHS: 4)	Nilgiris (MHS: 7)
1	*Haemaphysalis spinigera*	231 (17.7)	197 (32.8)	1 (0.25)	93 (13.3)
2	*Haemaphysalis turturis*	2	12	9	40
3	*Haemaphysalis bispinosa*	1	3	13	0
4	*Haemaphysalis intermedia*	2	1	0	0
5	*Haemaphysalis cuspidata*	1	0	0	0
6	*Ambylomma sp*	0	9	0	9
	Total	**237**	**222**	**23**	**142**

MHS: Man-hour spent; Figures in parenthesis denotes per man-hour density

1.5.1 Characterization of the bacterial toxins isolated from marine soil samples for the control of mosquito vectors

EM 1134: Oct 2011 – Sep 2014

Poopathi S

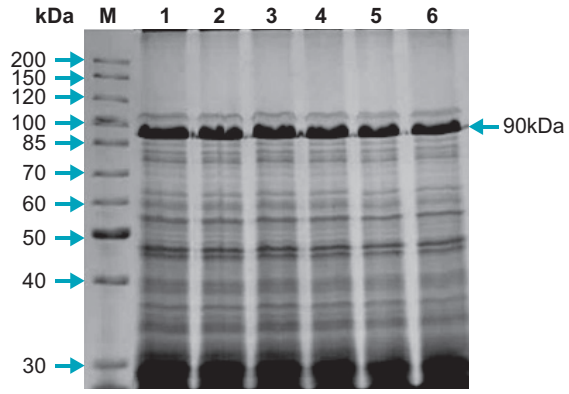
Introduction: Mosquito control is one of the most important public health objectives, as mosquitoes transmit many human diseases. These diseases pose a major health problem in disease-prevalent countries, and are presently wide-spread as a result of globalization, urbanization, and global warming. Mosquitocidal bacterial agents are environment friendly and therefore, there has been a tremendous effort worldwide to isolate efficient mosquitocidal bacteria. *Bacillus sphaericus* (*Bs*) and *B. thuringiensis* serovar *israelensis* (*Bti*) are the good candidature for mosquito control. Inspite of these, a high level of resistance to *Bs* has impeded the progress in its application in various countries. Under these circumstances, increasing attention has been devoted to discover new bacterial agents from the natural environment as an alternative to existing mosquitocidal bacteria. In the present study, we report for the first time a new bacterial agent, namely, *Bacillus cereus* VCRC-B540 (NCBI: JN377787) isolated from the gut region of marine red snapper fish (*Lutjanus sanguineous*) collected from the east coastal zone of the Bay of Bengal in the Union Territory of Puducherry (India) and show that it is a promising agent in controlling the mosquito vectors. Surface layer protein (90 kDa) was the responsible factor for this toxic effect.

Objectives:

- Screening of mosquitocidal bacteria from marine environment and characterization of mosquitocidal toxins.

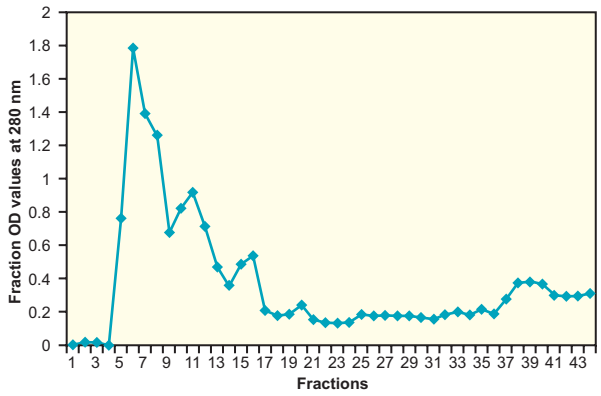
We have reported earlier a bacterium isolated from marine soil useful for mosquito control (AR-2012). In continuation of this work, an additional mosquitocidal bacterium isolated from the gut content of marine red snapper fish (Lutjanus sanguineous) was reported. 16S rRNA gene sequence alignment showed that this isolate belonged to the strain, Bacillus cereus VCRC-B540 (NCBI: JN377787). The growth pattern of B.cereus is on par with biomass production. SDS-PAGE show that the entire range of polypeptides were expressed in the region of 25 to 200 kDa and among these, the polypeptide which demonstrated evidence for toxicity against mosquito larvae (90 kDa) was distinct and conspicuous (**Figure 1.33**). Therefore, protein analysis strongly indicates that the toxicity is associated with the 90 kDa protein. For further confirmation, the bacterial strain was purified by Sephacryl S-200 column chromatography and each purified fraction was bioassayed against mosquito vectors. The result showed that the 7th fraction (OD at 280nm = 1.8) alone, out of the 43 fractions totally examined, showed toxicity (**Figure 1.34**). The characterization of this toxic protein from M/S MALDI-TOF sequence analysis revealed that it is a "surface layer protein (SLP)". This polypeptide revealed homology toward "SLP" (90 kDa) as shown in the protein mass fingerprint spectrum data here.

FIGURE 1.33 SDS-PAGE protein profiles of marine *Bacillus* cereus VCRC-B540 (JN377787)



Lanes: M = *Protein marker;* **1 to 6** = *Expression of mosquitocidal protein (SLP: 90 kDa) during different culture time (Hours: 24, 36, 48, 60, 66, 72)*

FIGURE 1.34 Sephacryl column chromatographic separation of mosquitocidal protein from *Bacillus cereus* VCRC B540 (JN377787)

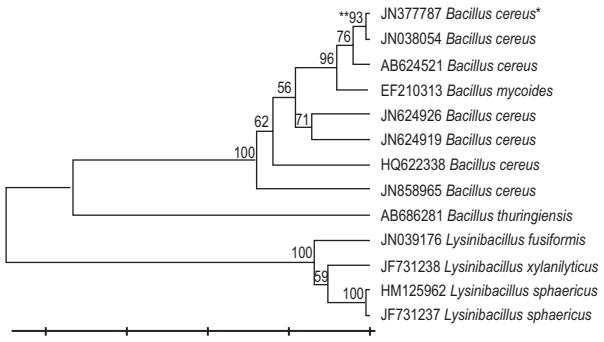


M: Protein marker, SLP = surface layer protein (mosquitocidal)

The red colour indicates corresponding polypeptide peaks matched information.

The homology of 16S rDNA gene sequence of the new *Bacillus* isolate (*B. cereus*) as investigated in the present study was compared with 16S rDNA gene sequences of closely related *Bacillus* strains from Genbank data base and a rectangular phylogenetic tree based on the topological algorithm was assessed. The result revealed that the 16S rRNA sequence reported in the present isolate is highly homologous to the species of *B. cereus* (**Figure 1.35**). The toxicity assays with *B. cereus* against the major mosquito vectors (*Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*) are shown in **Table 1.18**. It is observed that, *Cx. quinquefasciatus* is the most susceptible species, followed by *An. stephensi* and *Ae. Aegypti* that show moderate and lower susceptibility,

FIGURE 1.35 **Dendrogram showing the relatedness of 16S rDNA between the new isolate of *Bacillus cereus* VCRC-B540 (JN377787)* and selected reference isolates derived from Genbank database**



** Bootstrap values: statistical frequencies of new and reference strains

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  1 MAKQNKGRKF FAASATAALV ASAIVPVASA AQLNDFNKIS GYAKEAVQSL
 51 VDAGVIQGDA NGNFNPLKTI SRAEAATIFT NALELEAEGD VNFKDVKADA
101 WYYDAIAATV ENGIFEGVSA TEFAPNKQLT RSEAAKILVD AFELEGEGDL
151 SEFADASTVK PWAKSYLEIA VANGVIKGSE ANGKTNLNPN APITRQDFAV
201 VFSRTIENVD ATPKVDKIEV VDAKTLNVTL SDGTKETVTL EKALEPNKET
251 EVTFKIKDVE YKAKVTYVVT TATAVKSVSA TNLKEVVVEF DGTVDKETAE
301 DAANYALKSG KTIKSVSLAA DNKTATVTLT DKLNNNKADA ISISNVKAGD
351 KEINVKNVEF TAVDNKISEV TEVKSLGTKA VKVTLSEPVE HLSSTNFTLD
401 GKAYFGNVVM GAGNKTVILT PYSSSSALSVG DHKLTVSGAK DFAGFVSLNS
451 THEFKVVEDK EAPTVTEATA TLETVTLTFS EDIDMDTVKA SNAYWKSGDS
501 KKEASEFERI ADNKYKFVFK GSEKTLPTGK VDVYVEDIKD YSDNKIAKDT
551 KVTVTPEIDQ TRPEVRKVTA LDEKTIKVTF SKTVDGESAI KTGNYIVKDK
601 DDKVVSVDKV TVDSKDSKSVIIDLYSKVSV GENTITIKNV KDATKLNNTM
651 LDYTGKFTRS DKEGPDYEHV INADAKAKKV VLKFDKKMDA ASLADYSNYL
701 VKINDTLQTL SENVATLSVS NDATVVTITF AETIKGDDVV FASGKAISGS
751 GKVNVNELQV MGVKDTSGNV HKKFNGSENK ITLSSTSTPL KLAKIDKDYD
801 AKYTAELVDR KTVKVKFSTV INSAAANAFT SESHKIDSIQ VNGTSTVTAK
851 FKDEINTNAS DLDLKVNLSK LVDIAGNEST NNTPIAIKAG INLLDSVAPV
901 VVGEPVVDKE TITFTFSENL TSVSIGEVLS TDFTVTRVSD NKDLAIKDYS
951 VAIANNNQVV ITLSDNREVA TAYKVTAKNA KLITDDNGDK KNAIADFTKT
1001 TATKVEASGT LSLDAAKTNL NNEITKAKDA KATGTEGTAA TNQIVGSKDA
1051 LQVAIDVAEL V KNNTAATLQ QLTDAKTDLT AAITAYNAAK VEDISSLLVA
1101 PDLVLGTTVN GTITGYVAGT GETLKVTSDS AANVEVTDPT GLAVTAKAKG
1151 EANILVQVLK GDKVIKTGTV KVTVSE
```

respectively. The LC_{50} and LC_{90} values for *B. cereus* against *Cx. quinquefasciatus* were 0.004, and 0.037 mg/l, respectively.

From the foregoing study, it can be concluded that *B. cereus* isolated from the marine fish (*Lutjanus sanguineus*) is a potential candidate for the control of mosquito vectors, especially for the control of the filariasis vector of *Cx. quinquefasciatus*.

1.5.2 *In vivo* screening of six promising 1-N-methyl-4-(substituted) benzoyl/phenyl acetyl piperazides for macrofilaricidal activity against *Brugia malayi* in animal models

EM 1133: Feb 2012 – Jan 2015

Paily KP & Kalyanasundaram M (till Mar 2014)

In order to identify a compound with macrofilaricidal activity, six substituted phenyl acetyl/benzoyl piperazides were synthesised and they were found to exhibit moderate adulticidal activity against *Setaria digitata* under *in vitro* conditions (VCRC Annual Report, 2006). The present project is to test these compounds under *in vivo* conditions against adults of lymphatic filarial parasite, *Brugia malayi* (sub-periodic strain), using suitable animal models.

Objectives:

- To evaluate the promising six compounds *in vivo* against adult *B. malayi* in animal models in comparison with DEC citrate and
- To study the pharmacokinetics and toxicity of the effective compounds on host animals.

Out of the six compounds, which showed promising results under *in vitro* condition, the citrate salts of 1-N-methyl-4-substituted benzoyl piperazides (3-Methyl and 4-Methyl, coded as B_7 and B_8) only were effective against *B malayi* in mongolian gerbils (*Meriones unguiculatus*) (Annual Report, 2013). Hence, citrate salt of these compounds was synthesized further for *in vivo* testing in multimammate rats (*Mastomys coucha*). Citrate salts of 3,5-Dimethyl coded as B_{14}, the one which have not yet been tested in gerbil, was also synthesized for testing in gerbil as well as multimammate rats.

Institutional animal ethics committee clearance has been obtained for use of these animals for experiments. Mongolian gerbils were transplanted intra-peritoneally with 6 numbers of adult worms obtained from gerbils inoculated with infective larvae of *B. malayi*. Multimammate rats were infected with *B. malayi* by inoculation of 50

TABLE 1.18 **Toxicities measured by LC_{50}/LC_{90} values for *Bacillus cereus* VCRC-B540 (JN377787) against mosquito vectors**

Bacterial strain	Mosquito species	Intercept	Slope	LC_{50} (mg/L)* (90% UCL–LCL)**	LC_{90} (mg/L)* (90% UCL–LCL)	χ^2 (df)
Bacillus cereus (VCRC-B540)	*Culex quinquefasciatus*	8.30	0.61 ± 0.17	0.0047 (0.003–0.006)	0.037 (0.021–0.064)	1.68(7)
	Anopheles stephensi	8.60	0.72 ± 0.15	0.0068 (0.005–0.009)	0.04 (0.024–0.065)	2.29(6)
	Aedes aegypti	7.44	0.59 ± 0.16	0.016 (0.011–0.022)	0.14 (0.080–0.245)	2.16(6)

*Average performance of six individual observations.**90% confidential limits at upper and lower limits

numbers of infective larvae. Both for infection of gerbils and multimammate rats, the infective larvae were harvested from mf positive blood fed *Aedes aegypti* (Liverpool strain) mosquitoes.

After 7/8 days of transplantation of adult worms, two gerbils each were administered intra-peritoneally with the citrate salt of B_{14} at 100 mg/kg body weight consecutively for five days. Animals administered with normal saline were maintained as negative controls. The animals were routinely monitored for weight loss, food intake and body temperature. Animals were sacrificed 45 days after administration of the drug and examined for the presence of adult worms and microfilariae. The citrate salts of B_{14} caused 100% mortality of *B. malayi* adult worms (**Table 1.19**) as seen earlier with that of the B_7 and B_8. These animals exhibited remarkable level of reduction in mf density also.

The effective compounds, citrate salts of B_7, B_8 and B_{14}, which were found effective in causing mortality of adult worms in gerbils were further tested in *B. malayi* infected *Mastomys coucha* through oral administration of 200 mg/kg. Animals treated with B_7 and B_8 were sacrificed after 90 days and the worms alive were recovered. A total 7 worms could be recovered from B_7 treated animal. From B_8 treated animal, 17 worms could be recovered, indicating that both these compounds are not very effective as macrofilaricidal when given orally to infected animals. Animals treated with B_{14} could not be monitored as they died during the observation period, due to spontaneous tumor development which is a known inherent trait of multimammate rats. Hence, treatment with B_{14} was repeated on another two infected animals and they are being monitored.

Absorption of the drug after oral administration was studied in comparison with that of the DEC. Citrate salts of DEC and the test compounds were administered to multimammate rats orally at 200 mg/kg body weight. Blood samples were collected at 30 minutes interval and the rate of absorption was monitored through HPLC. DEC was at its peak level in the blood of the animal after 2 h of its administration. Blood samples from other drug treated animals are being analysed for their level of absorption. The results of this study will indicate whether it was because of low level of absorption of the B_7 and B_8 drugs they were not effective in killing adult worms in multimammate rats.

In vivo screening of citrates salts of B7, B8 and B14 will be repeated using each of 3 more numbers of *B. malayi* infected multimammate rats. Pharmakokinetics of these compounds are to be studied through analysis of blood samples collected from treated animals.

1.5.3 Development of new mosquito control agents based on anthranilic diamides targeting the insect ryanodine receptor

IM 1404: Apr 2014 – Mar 2017

Nisha Mathew & Jambulingam P

Development of insecticides with unique modes of action is necessary to combat widespread insecticide resistance. A new class of insecticides has been discovered, the anthranilic diamides, that provides exceptional control through action on a novel target, the ryanodine receptor. Anthranilic diamides potently activate this receptor, releasing stored calcium from the sarcoplasmic reticulum causing impaired regulation of muscle contraction. The diamides are the most recent addition to the limited number of insecticide classes with specific target site activity that are highly efficacious, control a wide pest spectrum, and have a favorable toxicological profile. Hence, the synthesis of substituted anthranilic diamides, mosquitocidal screening and structure activity relationship studies for the development of mosquitocidal agents will be taken up with following objectives:-

Objectives:

- To synthesis substituted anthranilic diamides.
- To evaluate the substituted diamides for larvicidal and adulticidal activity against the vector mosquitoes *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.*
- To study the structure activity relationship of the active molecule.

Eleven substituted anthranilic diamides (VCRC/ADA1-11) have been synthesized and purified by column chromatography. All the compounds were sent to Pondicherry University for obtaining ^{1}HNMR and ^{13}CNMR spectra and MS data. We received the spectral analysis data for three compounds which conforms the required chemical structures for the newly synthesized compounds.

Four compounds have been screened for mosquito larvicidal activity against early 3rd instar larvae of *Culex quinquefasciatus*. Two compounds showed 100% mortality at 100ppm at the preliminary screening experiment.

Further work to be carried out: (1) Synthesis and evaluation of substituted diamides for mosquitocidal activity. (2) Based on the preliminary data, a quantitative structure activity study has to be carried out using bioinformatic tools to get new molecules with better mosquito control potential.

TABLE 1.19 ***In vivo*** **antifilarial activity of Citrate salt of 3, 5-Dimethyl (B_{14} 100 mg/kg for 5 days) against transplanted worms of *B. malayi* in mongolian gerbils**

Compound (Replicate)	No. of worms transplanted	No. of worms recovered	% mortality of adult worms	Mf count in peritoneal fluid (20 µl)	Total mf count	Mf count in blood (20 µl)	Mf released/ female after recovery (for 1 h)
B14 (I)	6	0	100	280	17,500	2	0
B14 (II)	7	0	100	520	32,550	1	0
Control (I)	6	4	33.3	3,100	2,32,500	23	762
Control (II)	8	5	37.5	800	72,000	19	178

1.6.1 Development of naphthoquinone analogues as macrofilaricidal agents

IM 1306: Feb 2014 – Feb 2016

Nisha Mathew & Paily KP

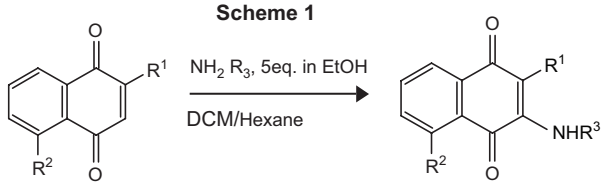
Currently, there is no effective adulticidal drug for the treatment of lymphatic filariasis. For the first time, VCRC has reported macrofilaricidal activity of a lead molecule, 5-hydroxy-2-methyl-l, 4-naphthoquinone (Plumbagin), isolated from the plant *Plumbago rosea/ indica (Indian Pat.* Appl.1083/DEL/2003). Subsequently for lead optimization, analogue molecules were synthesized with DST funding (DST No.SR/SO/HS-83/2005 dated 24-7-2007). Out of 75 compounds synthesized and screened 11 compounds showed promising *in vitro* macrofilaricidal activity against adult filarial worms (VCRC annual reports 2009, 2010 & 2011). The present project aims to identify the most promising naphthoquinone analogue among these, determination of drug like properties by *in vitro* ADME and to study its effect as macrofilaricidal agent.

Objectives:

- To identify the most active macrofilaricidal drug candidate from the selected 11 numbers of naphthoquinone analogues through assessment of metabolic stability and bioavailability of the molecules using *in-vitro* ADME screens.
- To study the macrofilaricidal effect of the most promising naphthoquinone analogue drug candidate through *in vivo* testing against the human filarial parasite, *B. malayi*, in an animal model, *Meriones unguiculatus*.

Out of eleven naphthoquinone (NPQ) analogues found promising in our earlier studies, six have been synthesized (scheme-1). The purified compounds were analyzed by FT-IR, ^{1}H and ^{13}C NMR. The spectral analysis showed that the synthesized compounds are having the required chemical structures.

Scheme 1



R^1 -H or -CH_3 or –OH; R^2 –H or -OH; R^3- H or alkyl or aryl

All the six synthesized compounds **TR-NPQ 1-6** were screened for macrofilaricidal activity by worm motility assay and MTT reduction assay to confirm the antifilarial activity of the lead molecules. The results are given in **Figure 1.36**. All the compounds exhibited *in vitro* macrofilaricidal activity in the preliminary screening at 0.1mg/ml.

Standard drugs with reported results have been studied along with NPQ analogues for the validation of each of the experimental methods except chemical stability studies.

The standard drugs (diclofenac sodium, caffeine, diethylstilbesterol and tamoxifen) with high solubility and poor solubility have been studied along with NPQ analogues for validation. The reported values for Caffeine and diclofenac sodium are >400µM and for Diethylstilbestrol and Tamoxifen the values are 5-20 and 3-30 µM respectively and the values obtained in our experiments are within the range of the reported values. Our studies show that the compounds TR-NPQ 1, 2, 4, 5 & 6 exhibit a solubility >100µM while TR-NPQ 3 has <50µM as shown in **Figure 1.37**.

Studies were carried out for determining the chemical stability in phosphate buffered saline (PBS), pH 7.4 at 25°C for 24 hours and at 37°C in simulated gastric fluid (SGF) for 2 hours and simulated intestinal fluid (SIF) for 4 hours which were prepared as per US Pharmacopoeia. The results of the stability studies are given in **Figure 1.38**. The stability study in PBS, pH 7.4, SGF and SIF revealed that the compounds TR-NPQ 1, 2, 4, 5 & 6 are stable at these conditions indicating its acceptability for intended absorption from the high surface area of the intestine. In the case of TR-NPQ 3 the stability at pH 7.4 after 24 hrs was slightly reduced however with SIF and SGF it was found to be stable.

Further work to be carried out: (1) The synthesis of TR-NPQ 7-11 and stability & solubility studies and *in vitro* macrofilaricidal screening of them. (2) Permeability, distribution coefficient, plasma protein binding and metabolic stability of TR-NPQ 1-11. (3) *In vivo* screening of the promising TR-NPQ for macrofilaricidal activity.

FIGURE 1.36 ***In vitro* macrofilaricidal activity of synthesized TR-NPQ 1–6**

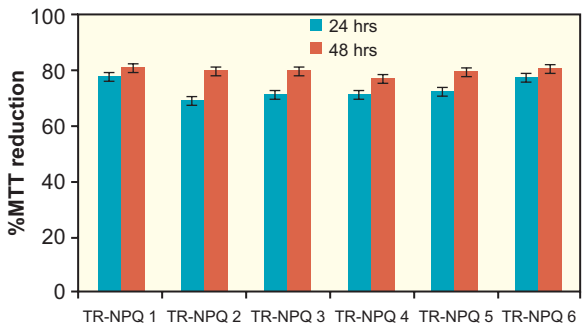


FIGURE 1.37 **Solubility of TR-NPQ 1–6 and standard drugs in PBS, pH 7.4**

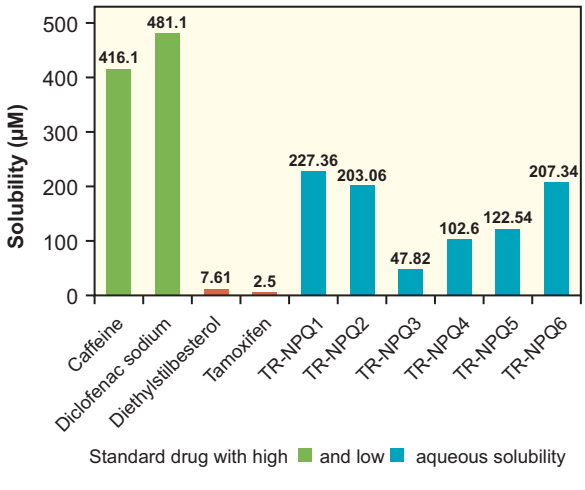
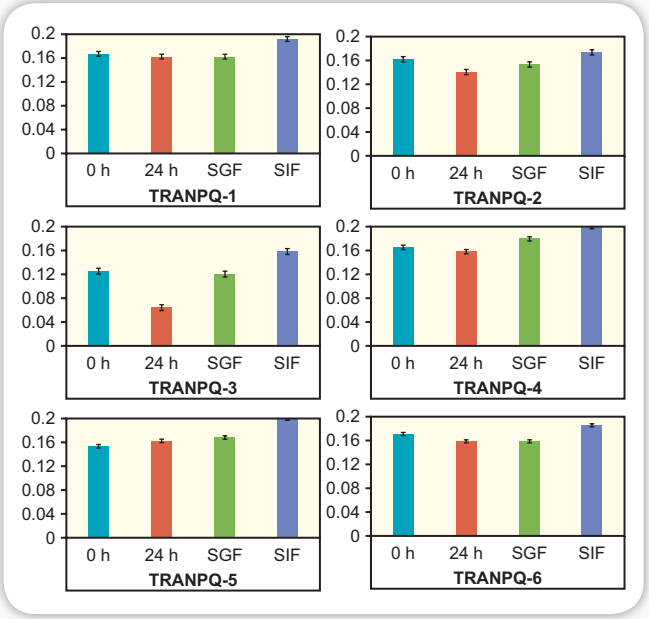


FIGURE 1.38 Chemical stability of TR-NPQ 1–6 in PBS pH 7.4, SGF and SIF



1.6.2 Optimization of upstream and downstream process for the production of mosquitocidal metabolite(s) by an indigenous bacterium *Bacillus amyloliquefaciens* and assessment of its anti-microbial activity

IM 1302: Apr 2013 – Mar 2016

Manonmani AM, Geetha I, Mathivanan A, Parija SC (JIPMER)

Secondary metabolite(s) produced by an indigenous strain of *Bacillus amyloliquefaciens* (B483) were found to have mosquito larvicidal and pupicidal activity. To develop this mosquitocidal bacterium as a biocontrol agent, the production processes *viz.* upstream and downstream need to be optimized for maximizing the yield of the metabolite. The crude mosquitocidal metabolite(s) was also found to show anti-bacterial activity against multi drug resistant (MDR) human pathogens. This property adds additional value to the biocontrol agent. Therefore, this project was initiated to optimize the production parameters, characterize the mosquitocidal metabolite(s) and study its antibacterial activity.

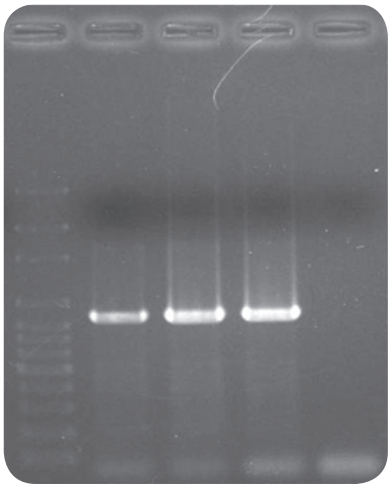
Objectives:

- To optimize the production parameters.
- To test the efficacy of the metabolite(s) against mosquito stages
- To purify and identify the mosquitocidal molecule
- To assess the anti-microbial effect of the crude and purified metabolite

An optimised production medium and downstream process for obtaining the metabolite by the mosquitocidal bacterium (VCRC B483) was achieved last year. Bacillomycin, a lipopeptide encoding bmyC gene (875 bp) was amplified and sequenced (KJ169570) (**Figure 1.39**). Phylogenetic tree based on protein sequence exhibited homology with mycosubtilin synthetase and Iturin synthetase. Amino acid composition revealed high percentages of Leucine, Aspartic acid and Isoleucine. The presence of valine at 55th position in our strain was found to be unique and different from the existing strains of *B. subtilis* and *B. amyloliquefaciens*.

Antibacterial activity of the metabolite(s) was tested against clinical isolates and highest activity was observed with *Staphylococcus aureus* (**Figure 1.40**). Further studies on MIC (minimum inhibitory concentration) determination and identification of the antibacterial molecule(s) are in progress.

FIGURE 1.39 PCR amplification of bmyC gene from *B. amyloliquefaciens* (VCRC B483)



Lane 1: 100bp molecular weight marker;
Lane 2–4: partially amplified bmyC gene with the size of 875bp;
Lane 5: Negative control

FIGURE 1.40 Zone of inhibition shown by metabolites against a clinical isolate of *Staphylococcus aureus*

1.7.1 Phase III evaluation to compare insecticidal efficacy and household acceptability of Icon Maxx, a long-lasting treatment for nets, with conventional insecticide treated nets in India

EM 1015: Jul 2011 – Sep 2014

Sahu SS, Gunasekaran K, Vijayakumar KN & Jambulingam P

Since new brands of potential long lasting insecticidal nets (LNs) and LN treatment kits require field evaluation before they are recommended for use in vector control programme, in the current study, evaluation, at Phase III level, of washed polyester nets impregnated with a new LN treatment kit, ICON MAXX, (hereafter referred as candidate LN) in comparison with polyester nets conventionally treated with the same insecticide, lambda-cyhalothrin (CS 2.5%) at the WHO recommended dosage of 15mg ai/m^2 (hereafter referred as ITN) was carried out in a *Plasmodium falciparum* endemic area of Odisha State in India.

Objectives:

- To evaluate the insecticidal activity and fabric integrity of washed polyester nets treated with ICON MAXX long-lasting treatment of nets over 36 months in comparison with conventionally treated mosquito nets using the same insecticide and under the same field conditions,
- To assess mode and habits of washing ICON MAXX treated nets and ITNs by the householders and
- To assess the community acceptability of ICON MAXX treated nets versus conventionally treated mosquito nets.

The evaluation was carried out in five selected villages, viz., Lendriguda, Ambaliambaguda, Kendriguda, Niraniguda and Bageipodar in Laxmipur Community Health Centre (CHC) of Koraput district, which is *in the southern region of the State and* predominantly inhabited by tribes.

This was a prospective study with nets as the unit of observation as well as randomization. In total, 440 households were included in the study. Of these, 300 households were given one coded LN each, and the remaining 140 households were given one coded ITN each. To cover the remaining persons in the households, 395 non-coded LNs were distributed. Consent was obtained in the selected villages to involve the community in the study. The required numbers of nets were treated one by one with ICON MAXX at 50 mg ai/m^2 to turn them into LNs. Similarly, polyester nets were treated with lambda-cyhalothrin CS 2.5% without the binder at 15 mg/m^2. A total of 835 nets were distributed to the households on 21st and 22nd July 2011.

A team of experienced field staff were trained on administering the questionnaire survey. Adverse effects of LNs/ ITNs, if any, among the net impregnators were assessed using a pre-designed questionnaire. Similarly, assessment of adverse effects, if any, among LN/ ITN users was made using a pre-designed questionnaire during the periodic surveys. At the end of week 1 and months 6, 18 and 30 post-net distribution, an adult member in each of the 30 selected households, from where nets were withdrawn for cone-bioassay, were interviewed visiting door-to-door (including early morning observations) to assess net utilization pattern/ frequency of use, method and number of washes, type of detergent used for washing and physical integrity of the net (size and number of holes) using a semi structured questionnaire. After 12, 24 and 36 months of net distribution, survey was done covering the households with the remaining coded LNs.

To ensure that the target dosage of the insecticide has been achieved, netting pieces were cut at the beginning of the trial for baseline assays and after one year of the trial. From each arm, 30 nets were destructively and randomly sampled for insecticide residue analysis.

After 36 months of household use, to confirm the availability of the insecticide on the used LNs, 50 nets were destructively and randomly sampled for chemical residue analysis. The residue analysis was done at the Phytopharmacy Department of the Agricultural Research Centre in Gembloux, Belgium (a WHO Collaborating Centre).

Insecticidal effect of ITNs/ LNs was evaluated after distribution of nets at one week / 2 months and every 6 months thereafter. IDs of 30 nets of each type (LNs/ ITNs) were randomly selected for cone-bioassays as prescribed by the WHO. LNs that caused a knockdown rate of $<95\%$ and/or a bioassay mortality of $<80\%$ were subjected to tunnel test.

At base line (at week 1 post-distribution), the average (95% CI) lambda-cyhalothrin content in ICON MAXX nets was 62.0 ± 7.4 mg/m^2 (59.2–64.8) with a range of 48.9 to 78.2 mg/m^2, which was slightly higher than the target dosage of 50 mg/m^2. In samples of conventionally treated nets, the average (95% CI) lambda-cyhalothrin content was 20.6 ± 2.8 mg/m^2 (19.6–21.6) with a range of 13.9 to 25.6 mg/m^2, which was also marginally greater than 15 mg/m^2, the target dosage.

One year after the distribution, relatively lower concentration of lambda-cyhalothrin than the target dosage was found in both ICON MAXX and conventionally treated nets. The mean lambda-cyhalothrin content has fallen to 45.2 mg/m^2 in the LNs corresponding to 71% of the baseline and to 13.7 mg/m^2 for the ITN corresponding to 64% of the lambda-cyhalothrin content at baseline.

After 36 months of household use, the mean (95% CI) content further reduced to 34.5 ± 20.4 mg/m^2 (28.7-40.3) with a range of 3.0 to 78.8 mg/m^2, which was lower than the target dosage of 50 mg/m^2. On 12 occasions, the content was more than 50 mg/m^2 with a range from 50.6 to 78.8 mg/m^2 and on rest of the 38 occasions, the content was <50 mg/m^2 with a range from 3.0 to 48.9 mg/m^2.

Bio-efficacy: In cone-bioassays up to 24 months, a complete (100%) knockdown of *An. stephensi* was noticed; the mean corrected mortality was 88.3% (85.8%-90.7%). Thus, all the nets tested in bioassays passed the WHOPES criteria. After 30 months, knockdown was 97.3% (95.3-99.4) and the corrected mortality was 67.0% (57.7%-76.3%). Out of the 30 LNs tested, 24 (80.0%) passed the WHOPES criteria as they caused $\geq 95\%$ knockdown and/ or $\geq 80\%$ corrected mortality. The remaining six (20.0%) nets were

tested in tunnel as per the WHOPES guidelines. In the tunnel tests, the average mortality was 34.5% (13.8%–55.2%) and the blood feeding inhibition was 30.7% (9.9%–51.4%), thereby the six nets failed to meet the WHOPES criteria. After 36 months of household use, overall, knockdown was 90.1% (88.5%–91.7%) and corrected mortality was 76.2% (73.1%–79.2%). Out of the 80 LNs on which bioassays were conducted, 45 (56.3%) passed WHOPES criteria. The remaining 35 (43.7%) nets were subjected to tunnel test and according to the results of the tunnel test, of the 35 nets, only two (5.7%) passed the WHOPES criteria (mortality ≥ 80.0% and/ or blood feeding inhibition ≥ 90%). The combined results of cone-bioassays and tunnel tests showed that out of the 80 LNs tested, 47 (58.8%) passed the WHOPES criteria (**Table 1.20**).

The lambda-cyhalothrin content at baseline was 62.0 ± 7.4 mg/m^2, which was reduced to 45.2 ± 15.8 mg/m^2 after one year of household use and after three years of use it was further reduced to 34.5 ± 20.4 mg/m^2. In cone-bioassays conducted after six months of net distribution, the mortality of *An. fluviatilis* was 100% and after three years, the mortality of *An. stephensi* against the LNs was 76.2%. The decrease of lambda-cyhalothrin content in the LNs after three years of household use was 55.6% of their baseline and there was a corresponding decrease in the mortality of the vector species from 100% to 76.2%. (**Table 1.21**).

At the end of the impregnation day and the following day of impregnation, of the six LN impregnators, four (66.7%) had complained of itching or headache or facial burning or nausea or body rashes or eye irritation that lasted for 24 hours. In the case of ITN, two of the six impregnators complained of such adverse effects lasted for the same duration. However, one week after the impregnation work, no one had any complaint.

One month after the distribution, while a few ITN users had complaint of itching, facial burning, sneezing, nasal discharge, and eye irritation etc., two LN users had a complaint of bad smell and body rashes. After six months of net distribution, only one person had a complaint of itching. At 12, 18, 24, 30 and 36 months after net distribution, none of the net users reported any adverse effect.

Physical inspection of nets: After 12 months of net distribution, 64 were found torn out of the 320 nets examined (240 LNs and 80 ITNs). Out of the 240 LNs, 42 (17.5%) were in torn condition. Mean number of holes per LN was 0.5; the mean (1SD) hole index was 25.3 (128.8) and the mean (1SD) hole area was 31.0 (158.1) cm^2. Most of the holes present in the nets were either in the lower half (58%) or in the upper half (30%) of the net and 12% was found on the roof. Out of 80 ITNs, 20 (25.0%) ITNs were found torn. Mean number of holes per LN was 0.7; the mean (1SD) hole index was 9.4 (36.8) and the mean (1SD) hole area was 11.5 (45.2) cm^2. Most of the holes present in the nets were either in the lower half (30%) or in the upper half (51%) of the net. The remaining holes (19%) were found in the roof of the net. After 36 months of net distribution, out of 300 LNs checked, 117 (39.0%) were found with holes of the size 1, 2 and 3. The mean number of holes per LN was 1.9. The mean (1SD) hole index was 109.1 (304.5) and the median (IQR) was 0

TABLE 1.21 % (No nets passing/no. nets tested) ICON Maxx & CTN meeting WHO efficacy criteria in cone & tunnel tests, and their combined pass rate

Survey (month)	ICON® Maxx LN		Lambda-cyhalothrin CTN	
	No. of nets	Mean conc. mg/m^2 (95% CI)	No. of nets	Mean conc. mg/m^2 (95% CI)
0	30	62.0 (59.2–64.8)	30	20.6 (19.6–21.6)
12	30	45.2 (39.3–51.1)	30	13.7 (9.3–18.1)
36	50	34.5 (28.7–40.3)	–	–

TABLE 1.20 % (No. nets passing / No. nets tested) ICON Maxx & CTN meeting WHO efficacy criteria in cone & tunnel tests, and their combined pass rate

Survey (month)	ICON® Maxx long-lasting treatment			Lambda-cyhalothrin CTN		
	Cone bioassays[a]	Tunnel tests[b]	Cone and tunnel tests combined[(a+b)]	Cone bioassays[a]	Tunnel tests[b]	Cone and tunnel tests combined[(a+b)]
0	100 (30/30)	–	100 (30/30)	100 (30/30)	–	100 (30/30)
6	100 (30/30)	–	100 (30/30)	100 (30/30)	–	100 (30/30)
12	100 (30/30)	–	100 (30/30)	100 (30/30)	–	100 (30/30)
18	100 (30/30)	–	100 (30/30)	–	–	–
24	100 (30/30)	–	100 (30/30)	–	–	–
30	80.0 (24/30)	0 (0/6)	80.0 (24/30)	–	–	–
36	56.3 (45/80)	5.7 (2/35)	58.8(47/80)	–	–	–

WHO criteria: [a]cone test: ≥ 80% mortality or ≥ 95% knockdown; [b]tunnel test: ≥ 80% mortality or ≥ 90% blood feeding inhibition where control tunnel test > 50% penetration into host chamber. Tunnel tests were carried out on nets that did not satisfy the cone test criteria.

(0). The mean (1SD) hole area was 133.9 (373.8) cm^2 with a median (IQR) of 0 (0). Almost equal proportion of holes were found in lower half (47%) and upper half (43%) of the nets and on the roof 10% of the holes were present.

A total of 300 LN holders were interviewed after 36 months of net distribution. Among them, 200 (66.7%) reported using the nets year round and every night, 62 (20.7%) using year round but occasionally and 16 (5.3%) using only seasonally. The interviews also revealed that 22 (7.3%) respondents reported not using the nets citing one or other reasons. The investigators verified the reliability of the statement given by the respondents on net usage by observing the position of the nets in the houses during the survey time. At the 36 months survey, majority of the nets (81.7%, n = 278) were found hanging either above the beddings or mattress. This could be an indirect evidence that nets were used by the people. However, a sizable percentage (18.4%, n = 278) of the people were keeping the nets inside boxes indicating seasonal or occasional use.

At the end of 36 months, out of 300 LNs examined, 268 (89.3%) were found washed. The mean (SD) number of washes was 8.3 (6.9) and this was the maximum compared to the earlier surveys. Out of the 268 washed nets, 265 (98.9%) were washed with commercially available detergent powder and the remaining three (1.1%) with detergent soap. All nets were washed in cold water; 171 (63.8%) were dried in sunlight, 84 (31.3%) in shade and 13 (4.9%) inside houses (**Table 1.22**).

At one month after distribution of nets, there was no loss of nets. At 30 and 36 months, the net loss was found to be higher, the maximum (12.6%, n = 564) was at 36 months.

Up to 30 months, the ICON MAXX LN met the WHOPES criteria (80% nets passed by combining the results of cone-bioassays and tunnel tests). However, at 36 months of household use, only 58.8% LNs met the WHOPES criteria. Since, >20% of the ICON MAXX LNs failed to meet the WHOPES criteria, the study was stopped at 36 months.

TABLE 1.22 Washing frequency and net appearance

Survey (month)	ICON® Maxx LLIN						Lambda-cyhalothrin CTN					
	No. of nets	Mean (SD) no. of washes	% General aspect of nets				No. of nets	Mean (SD) no. of washes	% General aspect of nets			
			Clean	*Slightly dirty*	*Dirty*	*Very dirty*			*Clean*	*Slightly dirty*	*Dirty*	*Very dirty*
0	30	0	83.3	13.3	3.3	0	30	0	73.3	23.3	3.3	0
6	30	0.9 (1.1)	40.0	36.7	23.3	0	30	0.7 (0.8)	53.3	33.3	10.0	3.3
12	240	1.8 (1.6)	40.9	33.8	21.9	3.4	80	1.8 (1.5)	32.9	31.6	31.6	3.8
18	30	1.7 (1.1)	23.3	63.3	10.0	3.3	–	–	–	–	–	–
24	180	4.8 (4.1)	19.4	52.9	18.2	9.4	–	–	–	–	–	–
30	30	4.4 (2.5)	26.7	16.7	50.0	6.7	–	–	–	–	–	–
36	300	8.3 (6.9)	22.4	35.7	31.3	10.7	–	–	–	–	–	–

1.8.1 Preparation and supply of insecticide impregnated papers (IIP) for determining susceptibility of vector mosquitoes to insecticides

EM 1307: Nov 2013 – Nov 2015

Nisha Mathew & Gunasekaran K

The National Vector Borne Disease Control Programme (NVBDCP), New Delhi, in India has been using insecticides of different chemical groups, organochlorine, organophosphorus and synthetic pyrethroids, for the control of vectors. Large scale use of insecticides in disease endemic areas since many years has resulted in development of resistance in vectors to certain insecticides and this warrants formulation of resistance management strategies. To formulate and implement such resistance management strategies in time, regular monitoring of vector susceptibility/ resistance to the insecticides under use is essential. Vector susceptibility tests are normally conducted in field using WHO tubes and insecticide impregnated papers at the diagnostic concentrations. Currently, the insecticide impregnated papers are imported from Malaysia. This project aims at establishing a facility at VCRC to prepare the insecticide impregnated papers (Made in India) and supply to NVBDCP for monitoring vector resistance/susceptibility to insecticides.

Objectives:

- To establish a National facility for supplying insecticide impregnated papers (IIPs) conforming to WHO standard for monitoring insecticide resistance/ susceptibility in malaria vectors to different insecticides used in the National vector control programme.

Technical grade organochlorine insecticide DDT was obtained from Hindustan Insecticide Limited, a Govt. of India Enterprise. Risella oil as a gift from Shell Company, Germany. Using the technical grade, impregnated papers (12x15cm) of DDT 4% were made in Risella oil (Shell) base and packed in labelled boxes. Each box contains eight impregnated papers. Control papers were made with Risella oil without the insecticide.

Technical grade Organophosphate insecticide Malathion was also obtained from the Hindustan Insecticide Limited. Impregnated papers of Malathion 5% were made with technical grade in Olive oil (Sigma) base and packed in labelled boxes. Control papers were made with Olive oil without the insecticide.

Technical grade synthetic pyrethroids were obtained from Tagros Chemicals India Ltd. Using technical grade insecticide, impregnated papers of Deltamethrin (0.05%), Permethrin (0.75%), Alpha cypermethrin (0.05%) and Lamda cyhalothrin (0.05%) were prepared in silicon oil (Sigma) base and packed in labelled boxes. Control papers were made with Silicon oil without the insecticide.

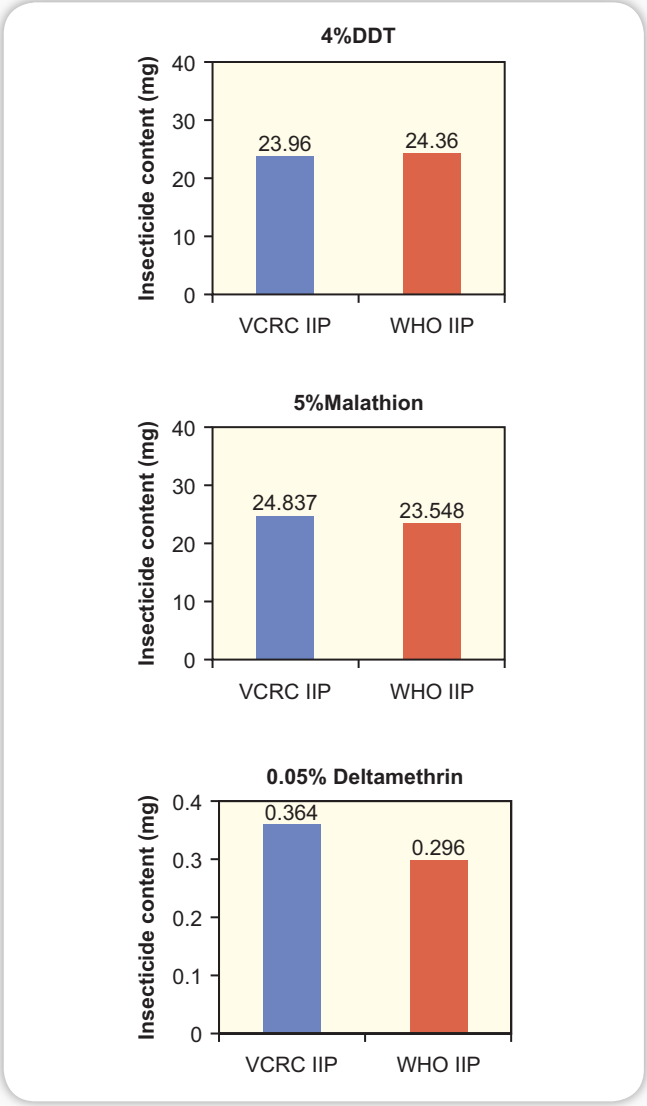
For a quality check the insecticide impregnated papers prepared at VCRC, along with WHO papers of the same insecticides were sent to National Horticultural Research and Development Foundation, Nasik, Maharashtra for the analysis of insecticide content (active ingredient). The GCMS analysis result obtained from Nasik is given in **Figure 1.41**.

The GCMS analysis shows that the quality of the insecticide impregnated papers were almost similar to that of WHO papers in terms of insecticide content in the case of all the three representative insecticides belonging to three different insecticide classes *viz.*, DDT (Organochlorine), Malathion (Organophosphorus) and Deltamethrin (Synthetic pyrethroid).

The shelf life of the impregnated papers is monitored through bioassays at VCRC at different periods post-preparation and compared with that of WHO papers.

Further work to be carried out: (1) Shelf life study of the impregnated papers. (2) Supply of insecticide impregnated papers to NVBDCP as per their requirement.

FIGURE 1.41 **Insecticide content analysed by GC-MS in VCRC-IIPs and WHO-IIPs**



1.8.2 Biomedical Informatics Centre of ICMR

EM: Jan 2014 – Dec 2018

Pradeep Kumar N, Gunasekaran K, Subramanian S, Nandakumar Y, Jayakodi G & Thulasi Babu R

Introduction: ICMR's Biomedical informatics centre (BMIC) at Vector Control Research Centre has been carrying out scientific activities to impart and provide solutions for biomedical research with informatics as a tool since January 2014. This is one among the 19 biomedical informatics centres established by ICMR and carrying out activities independently. This centre is closely associated and doing collaborative works with diversified scientific issues of the centre such as development of diagnostic kits for filariasis, insecticide resistance in malarial vectors, multidrug resistance in malarial parasites and identification of potential targets towards development of anti parasitic drug. The centre also operates in developing a novel database aiming to offer comprehensive data on genomics and proteomics of the arthropod vectors of public health importance in India. As a public reach out activity, the BMIC has designed a tutorial sort of website that holds facts about vector borne diseases including world perspectives and current scenario. The specific activities carried out by the BMIC during the reporting period are summarized below:

Objectives:

1. Development of vector informatics (VectorInfo) database
2. Role of *kdr* mutations in voltage gated sodium channel (VGSC) in conferring target mediated resistance to DDT and synthetic pyrethroids in malaria vectors
3. Prediction and evaluation of antigenic determinants on proteins of *Wuchereria bancrofti*
4. Identification of mechanism(s) of drug resistance (MDR) in human malarial parasites.
5. Pharmacophore based identification of inhibitors against Glutathione synthetase, a potential drug target of *Plasmodium falciparum*.

1. Arthropod vectors such as mosquitoes, ticks, mites, flies and fleas pose serious threat to humankind. Knowing the biology and molecular aspects of them is inevitable to control the diseases transmitted by them. Hence, structuring a database with open source access has become much more important, another critical issue lies in getting necessary clues of insect borne pathogens that are transmitted by bridge vectors. This current scheme outstretches to compile all data in one single window and organize for future access. To make this happen, designing VectorInfo (informative website) and VectorInfo db (all-inclusive database) has been commenced.

   Educative facts with reference to all vector agents were collected and grouped into i) Vector borne diseases of anthroponotic transmission and ii) vector borne diseases of zoonotic potential. Furthermore, diseases transmitted by vectors are independently ordered in terms of respective vector species and their diversity, causative organism, geographical distribution and drug/therapeutic agents reported to treat the disease. Innovate website with distinctive features was the result of the attempt made to portray respective accounts of mosquitoes, ticks and other insects in a channelized way (**Figure 1.42**).

FIGURE 1.42 Home page of Vector Informatics web resource

To develop the VectorInfo database (Vector Informatics data-base), a comprehensive survey of existing databases for each vector was carried out and the lacunae with the available sources were identified, ultimately aimed in providing an improvised and open source database offering all possible information regarding invertebrate vectors for the research community. The VectorInfo has been developed to deposit or to get access to the required data that are cornerstone features of vectors such as lifecycle, morphology, taxonomy, bionomics, ecology, vectorial capacity, role in the disease transmission and amenability to control. And furthermore separate emphasis is given to organize the genomic, transcriptomic, proteomic and metabolomic data of the biological weapons fighting against the humans.

Concerning genomic and proteomic data, the genomic sequence analysis including identification of gene elements, exons and introns, regulatory elements (promoters, operators, TFBs, TSSs etc), coding and non-coding regions, ORF regions, genome survey sequence, SNP sequence, biomarker sequence, similarity search, functional annotations are performed for all vectors. In addition, the pathogenic and non-pathogenic proteins are identified, sequences and structural analysis such as primary, secondary and tertiary structure prediction, validations, binding site identification and characterization and homology search are performed to intensify the data organization. An illustrative design to database homepage was finalized. Biological information of individual disease vectors was fed to the respective pages of the database (**Figure 1.43**). General information of mosquito and sandfly vectors have been incorporated into the database and search for other details are continued.

2. Any mutation will have its own specific impact on the conformation of protein irrespective of its location. Such conformational variations will contribute to deviated properties of the protein that lead to development of resistance to insecticides such as DDT/ Synthetic pyrethroids. But the underlying mechanism that confers the insecticide resistance due to mutations is to be delineated. Hence the current study aimed to predict the molecular mechanism of insecticide resistance using molecular modelling method with which the specific impact of any mutations can clearly be studied. This study reveals the variation in the conformation of the protein in domain and non domain regions also. The mutations will mainly affect the binding of the insecticides both in affinity ranges and orientation modes which will finally contribute to the resistance against the specific insecticide. To find out these implications and the underlying mechanism we followed the potent *in silico* approaches like molecular dynamics and docking studies.

The protein sequence of VGSC of *An. gambiae* was retrieved from Uniprot database which is of 2118 residues. An initial proteomic analysis was done using Protparam tool to know the physicochemical parameters of the channel protein and also the secondary structure information was predicted using SOPMA method. The trans-membrane helices were predicted using TMHMM tool. Since, suitable template to construct homology model of VGSC was not available, we have chosen *de novo* modelling process. The complete protein sequence was broken down into 6 segments and each segment was submitted to CABS de novo modelling server. The obtained models were joined together using Modeller tool and the final model was generated (**Figures 1.44 & 1.45**). This

FIGURE 1.43 **Digital database of insect vectors- VectorInfo (A repository of Indian vectors of human pathogens)**

FIGURE 1.44 The de novo modelling protocol for the construction of VGSC model

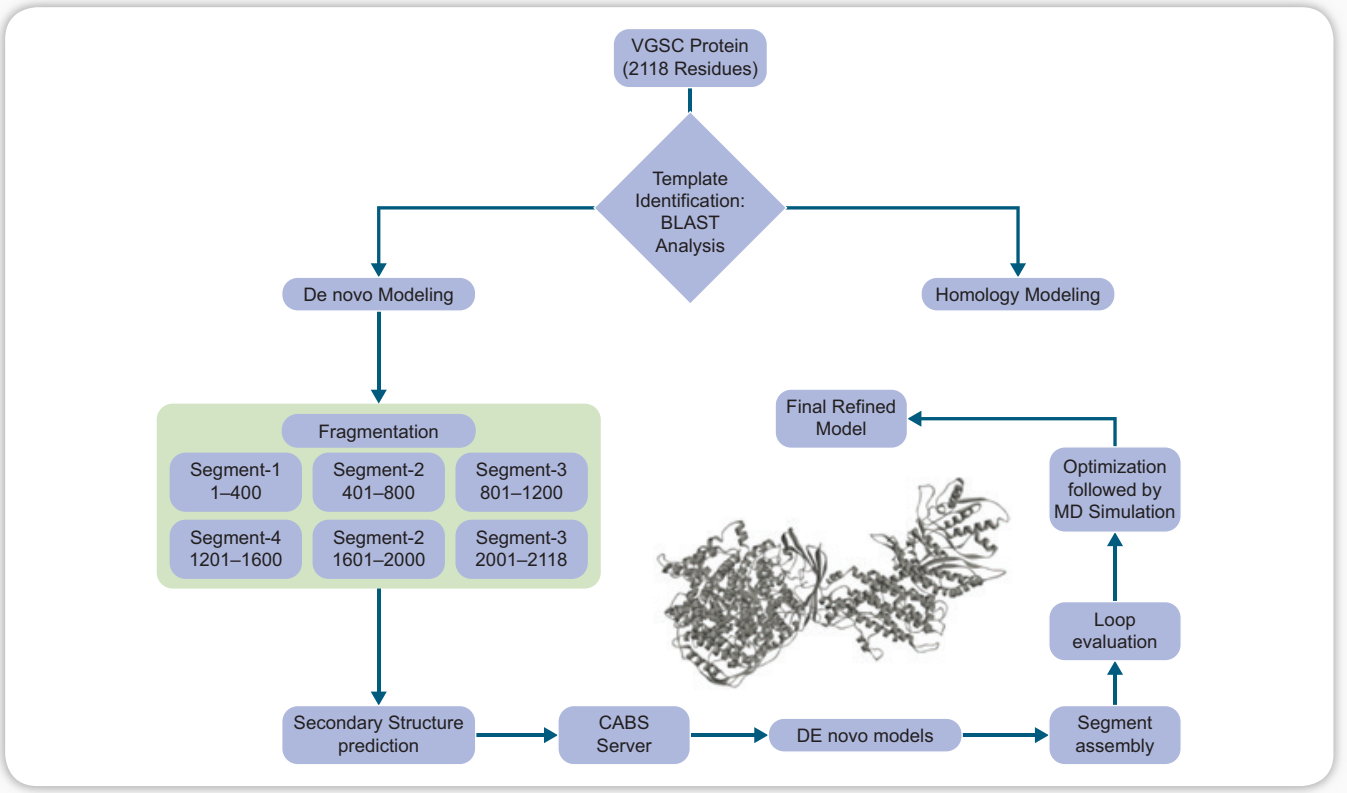
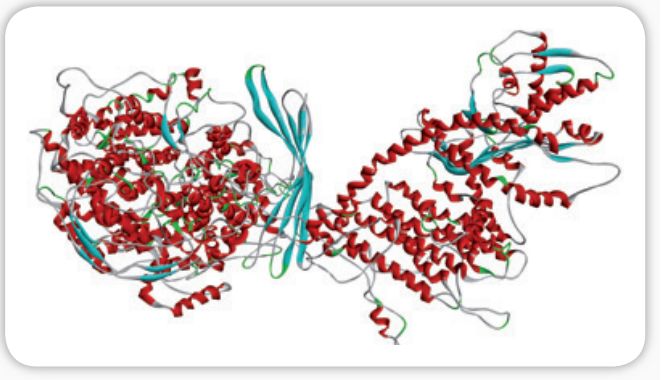


FIGURE 1.45 *De novo* model of Voltage gated sodium channel of *An. gambiae*



model was protonated and energy minimized and now the next task is to observe the behaviour of this model through simulations using molecular dynamics studies.

3. Lymphatic filariasis is a severe incapacitating disease of tropical and sub-tropical regions. Despite the availability of potent control methods and implication of different elimination programs for filariasis, complete knowledge of immunology of the parasite is yet to be understood and a challenge to the scientific community to move further since the pathogen encounters a different mechanism to bring about immune - modulation in the host immune system. The prediction of epitope regions on the proteins of *W. bancrofti* has a lot more patterns from the late 1970's but still the prediction of antigenic determinants is a question, hampered by the fact that very fewer information of genomic and proteomic data are accessible.

Understanding the antigenicity of a specific protein from *W. bancrofti* is of fundamental importance for the development of better, effective and more specific diagnostic tools. The availability of both sequence and structure of a protein makes the prediction of epitopes more effective and reliable. The three dimensional structural information helps to determine which areas of the sequence come into close enough proximity to form an epitope. The available sequence information of the present recourses can be made utilized to construct the theoretical three dimensional models of the proteins which will assist in determining perfect epitopes. Recently, several web based servers have become available that utilize both structural and sequence information to identify epitopes on the surface of a protein. The potential epitopes have the ability to induce a strong immunogenicity in the host which will be identified through *in vivo* evaluation after their synthesis. Even more a specific antibody can also be developed for the identified epitope regions. Optimization, synthesis and testing them *in vitro* will finally pave a best platform for the development of specific diagnostic tools for the detection of *W. bancrofti* infection.

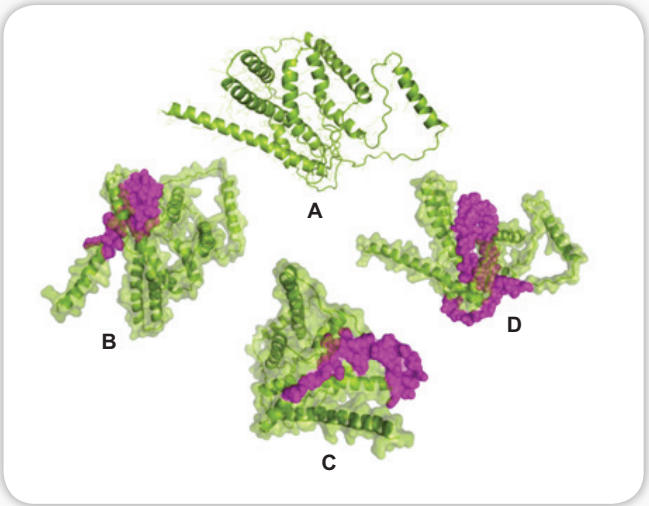
This concept was implemented initially taking cuticular collagen (CC) as starting protein. Based on the complete protein sequence, the *de novo* model of CC was constructed and the model has been validated for its stereo chemical quality. Later the protein sequence has been submitted to various antigen prediction tools and the obtained epitope regions were aligned with the complete CC sequence. Based on the alignment studies three peptides were finalized as potential antigenic regions (**Table 1.23**). The three epitopes were located in the CC structure to analyse their accessibility for antibodies (**Figure 1.46**). This information can be used to develop the assay protocol. Further these epitope regions will be synthesized and tested *in vitro* for their immunogenicity. Steps are in progress to design specific antibodies against these epitope regions.

4. Identification of the genes potentially involved in drug resistance and molecular markers related to drug resistance would provide a framework for studying the incidence and spread of drug-resistant human malarial strains. The multi drug resistant (MDR) gene has been identified by researchers, but the drug resistance mechanism is not defined so far. Hence, in this study, it is attempted to predict the resistance mechanism of MDR gene due to SNPs. It is aimed to construct the theoretical models of the target proteins and simulate its behaviour under specified conditions. They will act as basic tools to generate the mutant models where the observation of these mutant models and the correlation of their activities with wild model will reveal the impact of mutations on the protein conformation and how they confer resistance. The conformational studies clearly explain the impact of SNPs on the native conformation of the MDR protein especially in the drug binding sites. The molecular modelling studies will also help to identify the variable drug binding orientations with the MDR protein which are the critical factors of resistance.

TABLE 1.23 The predicted epitope regions of Cuticular collagen

S. No.	Epitope	Region (length)	Sequence
1	CCEP1	21–57 (37)	TRTQVLSKVNGELR SRRQAGYDSYQMG EGVRSVGVSH
2	CCEP2	77–113 (37)	TRTQVLSKVNGELR SRRQAGYDSYQMG EGVRSVGVSH
3	CCEP3	120–191 (72)	GGVTGAPSAPGGG CCGCGVSPPGPPG EPGPDGNDGANGE PGPPGKDGQDAPQ EAPTQPHIEWCFDC PDAPAG

FIGURE 1.46 A, The *de novo* model of Cuticular collagen. The predicted epitopes are located in CC model and shown as magenta coloured spheres i.e. B, CCEP1; C, CCEP2; D, CCEP3



The gene and protein sequences of MDR gene of *Plasmodium vivax* were retrieved from NCBI database. The primary and secondary analysis of the protein sequence was carried out to find out its physico-chemical properties and secondary structural information. The trans-membrane helical regions were predicted using TMHMM tool. The homology model of the MDR was constructed using Modeller tool taking crystallographic structure of multi-drug transporter of *Caenorhabditis elegans* as template. The stereo chemical quality of the model was validated and energy minimized (**Figure 1.47**). The optimized conformation of MDR was used for the mutation analysis where the mutations Y976F and F1076L have been identified from literature survey and they were located in the complete protein. The optimised conformations of wild type and mutant MDR models were analysed for their conformational variations through PDBSum analysis. Variations were observed only in terms of helix-helix

FIGURE 1.47 Homology model of MDR protein of *P. vivax* constructed using Modeller tool

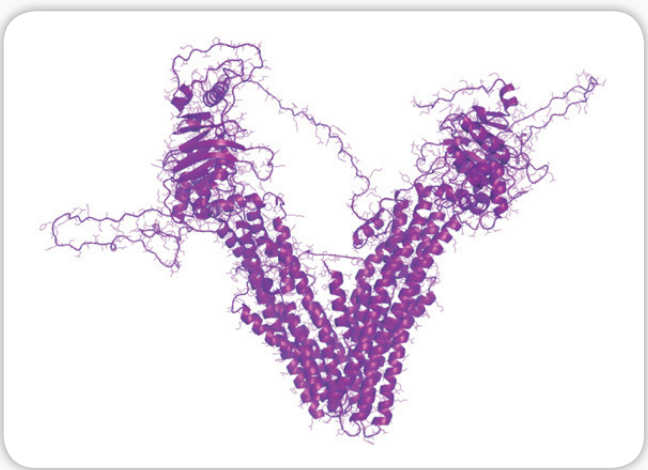
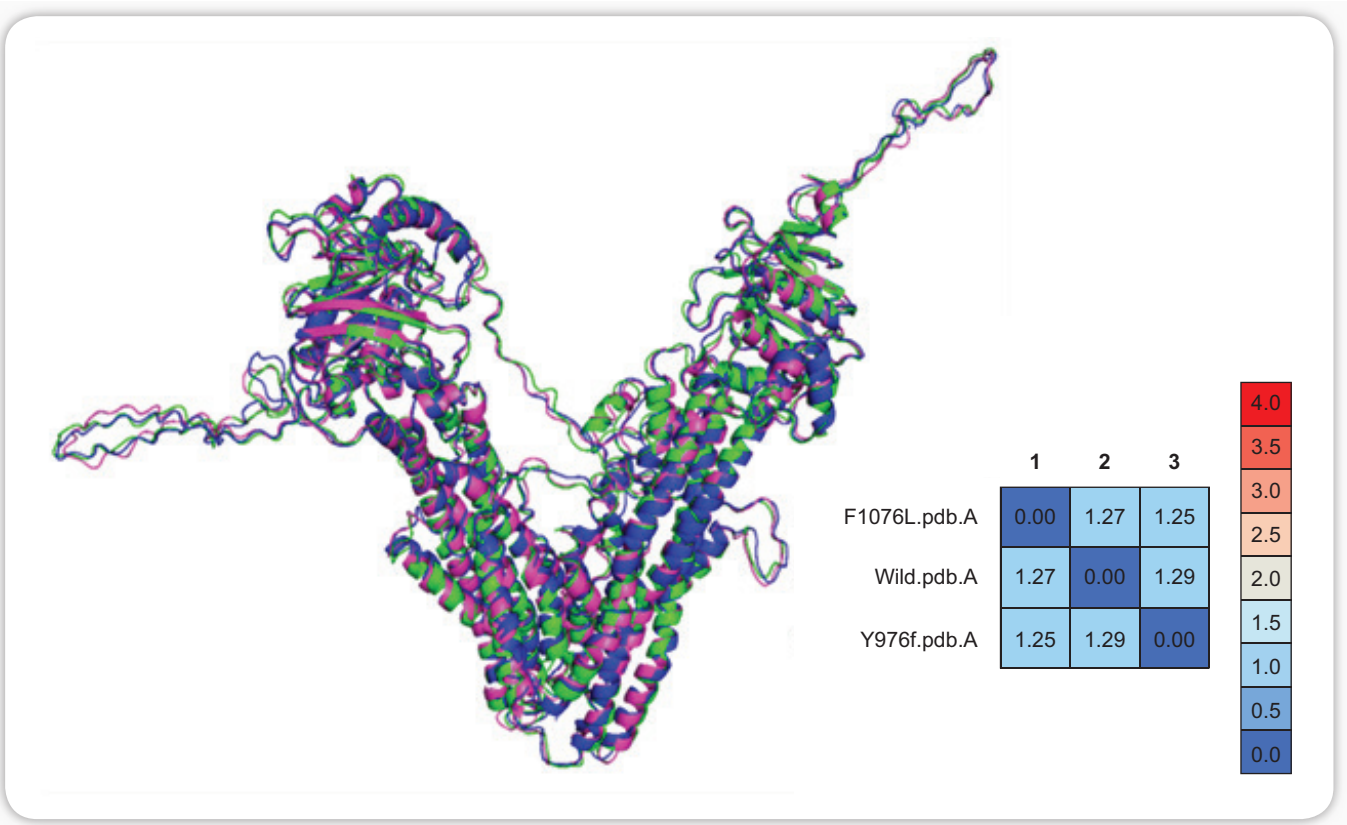


FIGURE 1.48 **Superimposition of Y976F and F1076L mutant models with wild type MDR model and the RMSD matrix indicating the RMSD variations among the wild type and mutant MDR models**



interactions and β-turns and there are no considerable variations in the secondary conformational elements like β-sheets, β-α-β units, β-hairpins, β-bulges, strands and γ-turns. Superimposition of the three MDR models revealed RMSD variations where the superimposition of Y976F mutant MDR showed an RMSD value of 1.29Å and F1076L showed 1.27Å which will explain the backbone fluctuations (**Figure 1.48**). These variations are to be investigated further through a long run of molecular dynamics studies to give more clear conclusions.

5. *Plasmodium falciparum* is a human malarial parasite causing significant morbidity and mortality throughout the world. However, the manifestation of drug resistance mechanisms in the parasite making its control more devastating. Hence it demands a rapid need to identify and validate new drug targets in the parasite metabolism. Glutathione synthetase (GS) is one of the potential drug targets of *P. falciparum* and is the subject of the present study where the inhibition of its metabolism leads to the death of parasite. Identification of glutathione structural analogues as GS inhibitors through computational means involving the clustering of library of compounds based on similar chemical features and derivatization of pharmacophore models that are complementary to the functional groups of GS binding site is rationally an impressive and attractive approach. Based on this we aimed to identify an effective inhibitor through pharmacophore modelling and virtual screening methods.

Initially *de novo* model of GS was constructed and validated (**Figure 1.49**) and subjected to energy minimization and molecular dynamics through standard dynamics cascade of Discovery studio software for a period of 10 nano seconds. The binding site was predicted based on the similarity with other available GS structures from the protein data bank. The structural analogues of glutathione were retrieved from PubChem data base and filtered using Lipinski rule and ADME

FIGURE 1.49 ***De novo* model of Glutathione synthetase of *P. falciparum***

properties. Two common feature pharamcophore models were derived from the final list of ligands (**Figure 1.50**) and their molecular interactions and affinity with the GS binding site were studied through molecular docking using LidDock module. Molecular docking study revealed that the compound CMBMB is showing better interaction and binding orientation in the GS binding site with best docking score when compare to the standard drug Chloroquine (**Figure 1.51**). Based on these observations it is concluded that, in view of non-toxicity, drug likeliness and favourable pharmacokinetic properties, CMBMB is identified as a safer drug molecule and further its efficient interactions with GS binding site promotes it as an effective lead to inhibit GS thereby glutathione metabolism which finally kills the parasite in host.

FIGURE 1.50 Common feature pharamcophore models of glutathione analogues

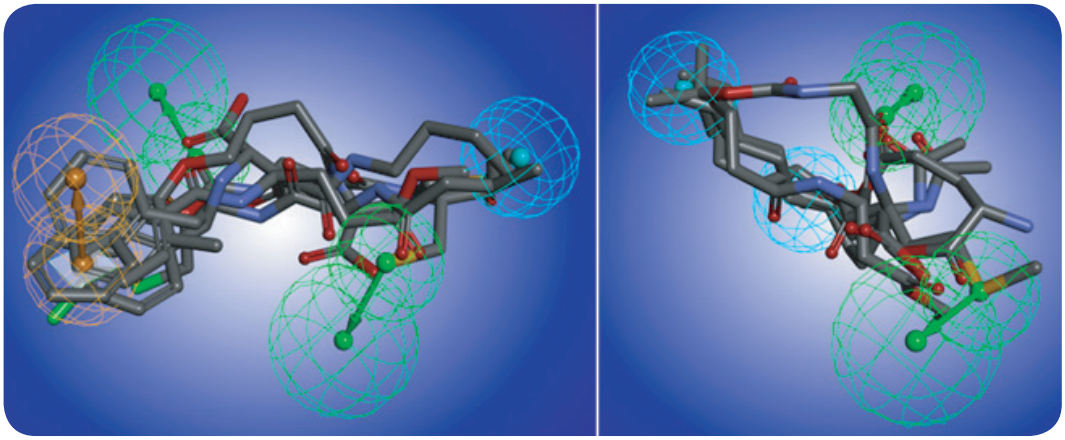
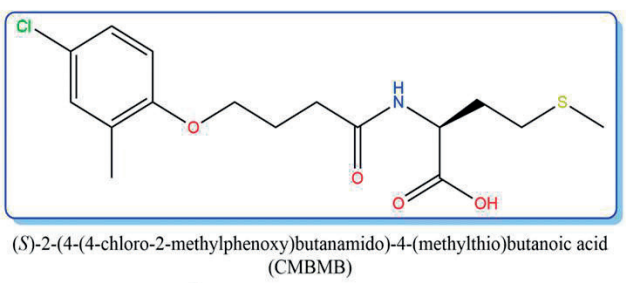
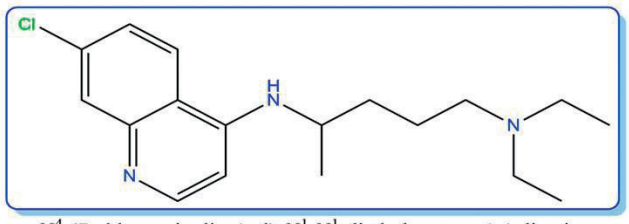


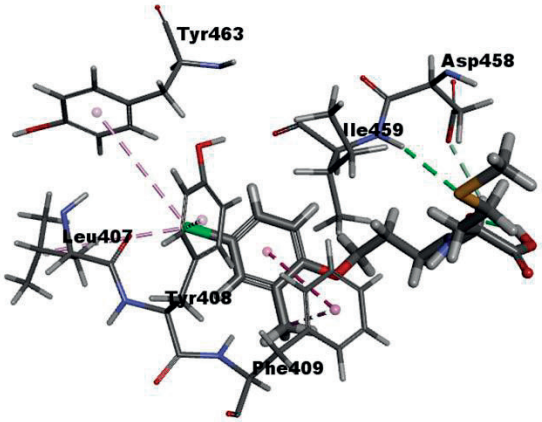
FIGURE 1.51 Two dimensional representations and binding mode orientations of CMBMB and Chloroquine in the binding site of GS

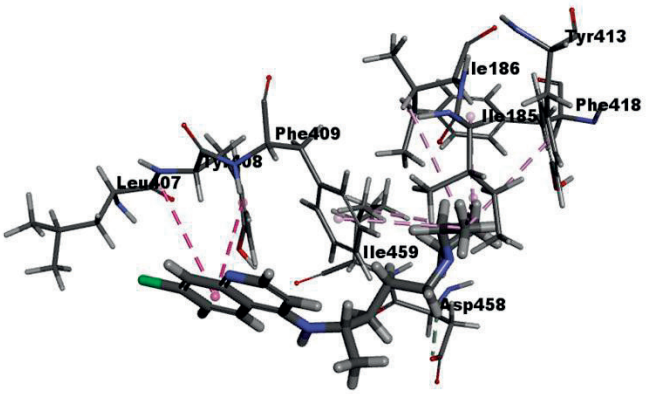


(*S*)-2-(4-(4-chloro-2-methylphenoxy)butanamido)-4-(methylthio)butanoic acid (CMBMB)



N^4-(7-chloroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine (Chloroquine)





UNIT

2

Human Resource Development

CONTENTS

2.1 Higher Education

There is a growing need for entomologists in the field of Public Health in view of emerging and re-emerging vector-borne diseases in India and other tropical countries. Most of the posts of Entomologists in many States of India are lying vacant. Apart from the State Health Departments, National Vector Borne Disease Control Programme (NVBDCP), National Centre for Disease Control (NCDC), National Rural Health Mission (NRHM), National Urban Health Mission (NUHM) and ICMR Institutes (VCRC, CRME, NIMR and RMRC) require personnel with knowledge and expertise on epidemiology and prevention/control of vector borne diseases for their programmes pertaining to vector borne diseases. In view of this felt need, on the recommendations of SAG, ECD, ICMR, a two year M.Sc. Public Health Entomology (PHE) course has been initiated at this Institute under affiliation to Pondicherry University.

2.1.1 M.Sc. Public Health Entomology

Twelve candidates have been admitted for the year 2014-16, fourth batch of M.Sc. Public Health Entomology course affiliated to Pondicherry University with a stipend of Rs. 6000/- and Rs. 3000/- per month respectively. Among these, one candidate (from Maldives) is sponsored by WHO.

Four students from the M.Sc. PHE batch 2012-14, have been selected for internship based on the inter-se merit list obtained from Pondicherry University.

As a part of the course curriculum, students have undertaken a tour to visit National Vector Borne Disease Control Programme and National Centre for Disease Control, New Delhi and other ICMR Institutes (NIMR Field Station, Goa, RMRI, Patna), besides our Field Stations (Koraput, Odisha & Kottayam, Kerala) for observational training in the R&D activities and hands on training in the operational aspects of vector borne disease control.

2.1.2 Post Doctoral Fellowship

One Post Doctoral Fellow has successfully completed his research under the ICMR PDF programme (Chemistry).

2.1.3 Ph.D. Programmes

Seventeen full time (Zoology – 10; Microbiology – 5; Chemistry – 2) and two part time Internal (one each from Zoology and Microbiology) candidates continue to pursue their Ph.D. programme.

2.1.4 MPH Programme at JIPMER, Puducherry

As an active partner of the course, the VCRC has engaged in delivering lectures, laboratory and field demonstrations in the areas of environmental public health, epidemiology and control of vectors and vector borne diseases.

2.2 Visit

Students from different Institutes of Puducherry, Tamil Nadu and Kerala visited VCRC for orientation and exposure to various ongoing programmes of the centre and the details are as under.

S.No.	Name of the College/Institution	Course/Title	No. of Students
1	Indira Gandhi Medical College & Research Institute, Puducherry	MBBS	96
2	Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry	B.Sc. Nursing MHW	79 66
3	Pondicherry University Community College, Puducherry	Sanitary Inspector	22
4	Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry	MBBS	60
5	Sri Ramachandra University, Chennai	Master of Public Health	10
6	Nangelil Ayurveda Medical College, Ernakulam	Preventive and Community Medicine	55

PG students from Puducherry were offered training in the following areas:

S.No.	Trainee particulars	Field of Training	Period
1	MD Students (4 Nos), Dept. of Community Medicine, Pondicherry Institute of Medical Sciences, Puducherry	Epidemiological surveillance tool for VBDs, Control of mosquito borne diseases, Implement rapid response and disaster management w.r.t. VBD outbreaks	July 2014 (1 week)
2	MD Students (3 Nos.) Dept. of Community Medicine Sri Manakula Vinayagar Medical College and Hospital, Puducherry	Vector Control Methods	Feb 2014 (1 week)

Training Programme at VCRC Field Stations

Koraput, Odisha

S.No.	Trainee particulars	Field of Training	Period
1	Entomologists (2 Nos.), NVBDCP, Odisha	Field level training on entomological aspects of ongoing malaria control activities	21–22 Jan 2014
2	Sentinal Sites Malaria Technicians (2 Nos.) of Koraput district	Malaria microscopy	19–20 Mar
3	Assistant Entomologists (2 Nos.) & Insect Collectors (2 Nos.) of NVBDCP, Odisha	Entomological aspects of Malaria and other vector borne diseases	27 Oct
4	District, CHC/ PHC and SC level Health Department personnel (40 Nos.)	Microfilaria and antigenemia survey in connection with ELF programme in Jagatsinghpur, Odisha State	18 Jul
5	District, CHC/ PHC and SC level Health Department personnel (32 Nos.)	ICT in connection with the Transmission Assessment Survey in Koraput district, Odisha State	17 Sept

Kottayam, Kerala

S.No.	Trainee particulars	Field of Training	Period
1	District level Health officials (30 Nos.), Trichur District	Insecticide spray operation in Visceral Leishmaniasis reported village, Kondazhy	4–7 Aug
2	District health Officials (5 Nos.), Kottayam, Kerala	Filariasis pre-MDA TAS training program	13 Nov

2.4 National Science Day

National Science Day was celebrated on 14th March 2014 in T. R. Rao Auditorium, VCRC. Competitions were conducted for the Ph.D. Scholars and M.Sc. students in Essay writing, Elocution, Pencil Sketch, Chess & Carrom. Prizes were distributed to the winners.

2.5 Swachh Bharat Campaign

School students' poster competition

Hygiene/Cleanliness drive by VCRC Staff on 02-10-14

2.6 Networking

A Networking initiative with our medical public health entomology students

A meeting was held at VCRC, Puducherry on 26.12.2014, as an inaugural reunion of the alumni to discuss the possibilities of networking among the entomologists who had graduated from the institute and assess their placements. A total of 24 participants representing 14 Medical Entomology Post Graduates, 1 PG Diploma Medical Entomology and 9 Public Health Entomology Post Graduates attended the meeting. The founders of these academic programmes were also present. The scope of our PG programmes in relation to career prospects and the possibilities in promoting and supporting Vector Borne disease research in India was discussed during this meet.

UNIT

3

Services and Supplies

CONTENTS

3.1.1 Independent Appraisal of LF elimination programme in India

The National Health Policy of India in 2002 set the goal of achieving elimination of lymphatic filariasis and kala-azar by 2015. The National Vector-Borne Disease Control Programme (NVBDCP) launched the Programme for Elimination of Lymphatic Filariasis (PELF) in 2004. The Ministry of Health & Family Welfare, Govt. of India, conducted an independent appraisal of the programme in 2007, when the programme was in the phase of up scaling the Mass Drug Administration (MDA) in the districts and was focusing on improving drug coverage and compliance. Appropriate actions were implemented based on the recommendations of the appraisal. Over the years, there has been considerable progress and achievements. As the programme is close to the target year of elimination (2015), there is an urgent need for consolidating the progress and planning future course of action. In this context, the Ministry of Health, Govt. of India invited the Indian Council of Medial Research (ICMR) to conduct an independent appraisal of the LF elimination programme in India through the Vector Control Research Centre (VCRC), Puducherry. A draft protocol was developed by VCRC and finalized in a meeting at NVBDCP, Delhi. Six teams, consisting of national experts and scientists from ICMR institutions were formed and the appraisal was conducted in six selected States in July 2014.

3.1.2 Insecticide resistance in malaria vector in southern districts of Odisha

The current strategy to control malaria vectors in the southern districts of Odisha includes mainly IRS with DDT/ synthetic pyrethroids and use of long lasting insecticidal nets (LLINs). At the request of State Health Department, insecticide susceptibility of the vectors were assessed in these districts. A study was undertaken in five of the ten southern districts of Odisha State to determine the susceptibility/resistance status of *An. culicifacies* to DDT, malathion and deltamethrin, the commonly used insecticides. The study showed that *An. culicifacies* was susceptible to deltamethrin in two districts while in the other eight districts; its response to the insecticide was under 'verification required' category indicating its tendency towards development of resistance to deltamethrin.

Susceptibility status of *An. culicifacies* was studied in Rayagada, Nowrangpur, Kalahandi, Malkangiri and Koraput districts. Adult female mosquitoes were collected from cattle sheds and human dwellings in the study villages in the early morning hours using mouth aspirator and flash light. The papers impregnated with DDT 4%, malathion 5% and deltamethrin 0.05% were obtained from the University Sains Malaysia, Penang, Malaysia and susceptibility tests were conducted using WHO kits.

A total of 567, 567 and 606 fully fed *An. culicifacies* females were exposed to DDT 4 %, malathion 5 % and deltamethrin 0.05 %, respectively in all the five districts. Parallel controls were maintained for comparison. The corrected mortality of this vector species ranged between 11.4% and 15.3% against DDT 4%, 60.4% and 76.2 % against malathion 5% and 72.6% and 84.0% against deltamethrin 0.05% (**Table 3.1**).

The results, thus, showed that *An. culicifacies* was resistant to DDT, malathion and deltamethrin in all the five districts. Resistance management strategy by appropriate rotation of different groups of insecticides should be considered in the areas, especially where *An. culicifacies* is more predominant.

3.1.3 Usage pattern and insecticidal efficacy of PermaNet 2.0 (long-lasting insecticidal net) after 2 to 5 years of household use in Odisha State

At the request of the State Health Department, an updated information on with-holding insecticidal efficacy, usage practice and physical integrity of LLINs at different time intervals of field use was collected to rationalize its use and strengthen the programme. Since, no such information was available with the programme.

TABLE 3.1 **Response of *An. culicifacies* to DDT, malathion and deltamethrin in the five southern districts of Odisha state**

District	DDT 4%						Malathion 5%						Deltamethrin 0.05%					
	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response	Number exposed		Number dead		CM (%)	Response
	T	C	T	C			T	C	T	C			T	C	T	C		
Rayagada	135	58	17	0	12.6	R	130	66	82	0	63.1	R	131	55	107	1	81.4	R
Nawarangapur	105	63	12	0	11.4	R	110	55	78	1	70.4	R	113	64	82	0	72.6	R
Kalahandi	105	63	13	0	12.4	R	111	67	67	0	60.4	R	131	55	104	0	79.4	R
Malkangiri	111	62	14	0	12.6	R	105	63	80	0	76.2	R	119	51	100	0	84.0	R
Koraput	111	64	17	0	15.3	R	111	62	74	0	66.7	R	112	56	86	0	76.8	R

T - Test, C - Control, CM - Corrected mortality, R – Resistant.
As per the WHO criteria, a corrected mortality of >98 % is 'susceptible', <90 % is 'resistant' and 90-98 % is 'verification required'.

The study was carried out in Borigumma and Laxmipur Community Health Centres (CHCs) of Koraput district and in Khairput CHC of Malkangiri district. Three sub-centres (SCs) namely Sargiguda, Kadamguda and Panchada were selected for the study respectively from Borigumma, Khairput and Laxmipur CHCs. The LLINs were distributed in Sargiguda in November 2009, Kadamguda in May 2010 and Panchada in June 2012, as per the NVBDCP guidelines. In each of these three SCs, five villages were selected for carrying out sociological survey and bioassay. A two-stage cluster sampling design was followed for selecting the villages as the stage 1 sampling unit and household as the stage 2 sampling unit.

The sample size for the survey was estimated by assuming 40% use rate in the study area (with an error margin of 10%, and 95% CI) and adjusted for attrition rates of 15%, 10% and 7.5% considering 5, 4, and 2 years net usage in Borigumma, Khairput and Laxmipur CHCs, respectively. Thus, a total of 106, 100, and 103 households were selected for assessing the use rate.

In each selected village, household members were interviewed by door-to-door visit to assess the availability of nets in the households, net utilization pattern/frequency of use (including early morning observations), mode of washing and number of washes and type of detergent used, physical integrity (size and number of holes) and attrition rate of the net. The household head or any adult member present at the time of team's visit was interviewed using a semi structured questionnaire.

Cone bioassays were carried out using laboratory-reared 2 to 5 days old; non-blood fed F1 progeny of *An. stephensi* to determine insecticidal efficacy of the LLIN. In each village, six nets selected randomly were withdrawn and in total 90 PermaNets 2.0 were evaluated for residual efficacy. From each net, five samples (25 cm x 25 cm) were cut from positions 1 to 5 as specified by the WHOPES procedure for bioassays.

In total, 1406 persons (n = 7178) from the 309 houses (n = 1624) selected in the 15 villages of the three CHCs were surveyed/ interviewed. To the 309 houses, a total of 446 LLINs had been distributed at the rate ranged from 2.8 to 4.0 persons per net with an average of 3.2. Among them, a total of 102 (82.9% of 123), 124 (73.3% of 169) and 126 (81.8% of 154) LLINs were physically present in the study villages of Borigumma, Khairput and Laxmipur CHC, respectively (**Table 3.2**).

The percentage of LLINs under use was 74.8%, 50.3% and 47.4%, respectively in the three CHCs (**Table 3.3**).

In Borigumma CHC, a majority (76.1%) of the respondents reported washing their nets >20 times and the remaining <20 times, but in the other two CHCs, Khairput and Laxmipur, the maximum (95.3% and 91.8%, respectively) reported washing <20 times. All nets were reprtedly washed with cold water and dried under sun. Among the washed nets, 93.5%, 100.0% and 86.3% in Borigumma, Khairput and Laxmipur CHC, respectively, were washed with 'surf', a commercial detergent powder (**Table 3.4**).

Of the total 352 LLINs inspected for physical integrity, only 14.7 %, 24.2 % and 57.1% nets were in good condition (no holes) in Borigumma, Khairput and Laxmipur CHC, respectively and

TABLE 3.2 Number of LLINs provided and physically present during the survey time

Name of CHC	Borigumma	Khairput	Laxmipur
No. of Households surveyed	106	100	103
No. of nets provided	123	169	154
No. of nets present (%)	102 (82.9)	124 (73.3)	126 (81.8)

TABLE 3.3 Usage rate of LLINs in households

Name of CHC	Borigumma	Khairput	Laxmipur
Total no. of villages surveyed	5	5	5
Total population of surveyed villages	3462	1822	1894
Total households of selected villages	824	349	451
Total no. of households surveyed	106	100	103
Total population in HHs surveyed	489	471	446
No of nets supplied in HHs surveyed	123	169	154
Distribution @ person/net	4.0	2.8	2.9
No of nets under use previous night	92	85	73
Population using nets	230	213	183
% of LLINs under use previous night	74.8	50.3	47.4

TABLE 3.4 Washing frequency of LLINs in different CHCs

Number of Washes	Borigumma (n = 92)	Khairput (n = 85)	Laxmipur (n = 73)
0 to 5	4	30	45
5 to 10	5	18	9
10 to 15	4	7	8
15 to 20	9	26	5
<=20	23.9	95.2	91.7
20 to 25	14	4	4
25 to 30	30	0	2
30 to 35	5	0	0
35 to 40	21	0	0
>20	76.1	4.8	8.3

the remaining were found with holes. The proportion of nets with hole size 1, 2, 3 and 4 are given in **Table 3.5**.

The percentage of LLINs lost by all means (overall attrition rate) was 17.0%, 26.6%, and 18.2%, respectively in the three CHCs (**Figure 3.1 & 3.2**).

In total, 1500 *An. stephensi* females were exposed to 30 LLINs withdrawn from each CHC. Overall, the corrected mortality was 7.2%, 34.6% and 100.0% in Borigumma, Khairput and Laxmipur CHCs, respectively (**Table 3.6**). Regression analysis revealed that the corrected mortality declined with number of washings (**Figure 3.3**, r = – 74 and P = 0,002).

The community needs to be educated with appropriate health education tools that should be socially and culturally acceptable to the local community, for enhancing the usage rate and reducing the practice of frequent washing of LLINs. The report has been submitted to the state health department.

TABLE 3.5 Physical integrity of nets in study area

Holes	Borigumma (n = 102)	Khairput (n = 124)	Laxmipur (n = 126)
LLINs with holes (%)	87 (85.3)	94 (75.8)	54 (42.9)
LLINs without holes (%)	15 (14.7)	30 (24.2)	72 (57.1)

TABLE 3.6 Results of bioassay

CHC	No. of nets tested for bio-assay	Range of the corrected mortality (%)	No. of nets	Average corrected mortality (%) with 30 nets
Borigumma	30	0	14	7.2
		1 to 20	14	
		21 to 40	0	
		41 to 60	2	
		61 to 80	0	
		81 to 100	0	
Khairput	30	0	0	34.6
		1 to 20	6	
		21 to 40	15	
		41 to 60	7	
		61 to 80	2	
		81 to 100	0	
Laxmipur	30	0	0	100.0
		1 to 20	0	
		21 to 40	0	
		41 to 60	0	
		61 to 80	0	
		81 to 100	30	

FIGURE 3.1 Map showing the study villages in the three CHCs

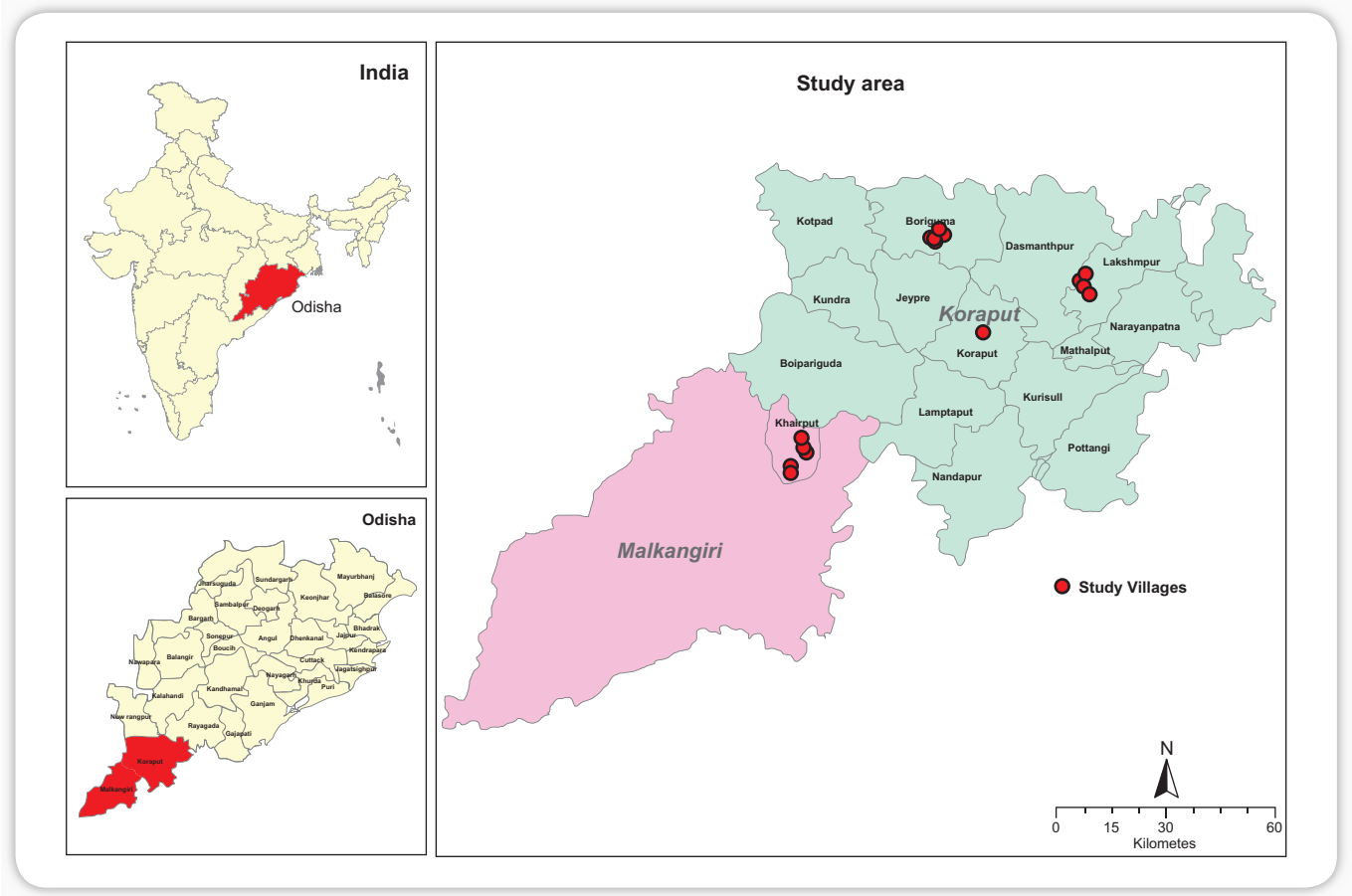


FIGURE 3.2 LLINs attrition rate in the three CHCs

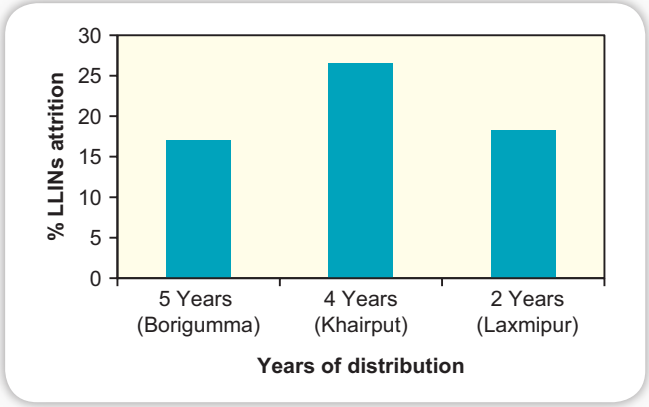
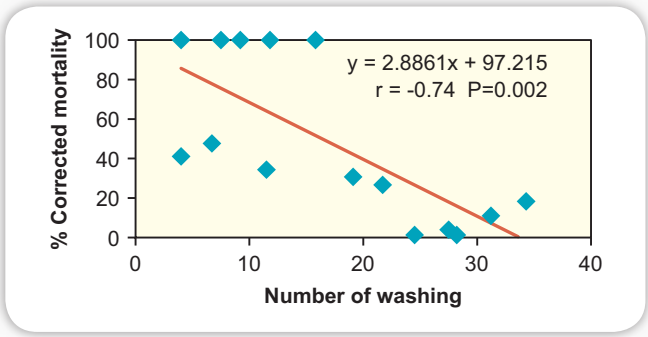


FIGURE 3.3 Relation between % mortality of *An. stephensi* and number of wash per net



3.2.1 Clinical services

VCRC filarial clinic provides diagnostic, therapeutic and morbidity management services to lymphatic filariasis patients reporting from Puducherry and field practice areas of Tamil Nadu. The numbers of patients who have attended the clinic is given in **Table 3.7**. A total of 25 patients were referred to GH and JIPMER for advanced treatment. E-advice on diagnosis and management was provided to 8 patients. Serological examination with ICT kit and haematological investigations were done for 8 and 6 filariasis patients respectively. Random Blood Sugar was checked for 17 patients. Post graduate physiotherapy students of MTPG & RIHS, Puducherry provided physiotherapy services to 16 lymphatic filariasis patients.

TABLE 3.7 Number of patients who availed the clinical services during Jan'14–Aug'14

Clinical Diagnosis	Number of Patients		
	First Visit	Repeat Visits	Total Visits
LE Grade-I	3	9	12
LE Grade-II	32	301	333
LE Grade-III	17	287	304
LE Grade-IV	11	111	122
Acute Adenolymphangitis	5	24	29
Others*	16	19	35
Total	**84**	**751**	**835**

** Others include non-filarial skin infections, trauma etc.*

3.2.2 Laboratory animal facility

The Centres' laboratory animal facility has breeding colonies of animals such as BALB/c mice (*Mus musculus*), mongolian gerbils (*Meriones unguiculatus*), and multimammate rats (*Mastomys coucha*). The facility is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals are being used for various ongoing research projects after getting approval of the Institutional Animal Ethics Committee (IAEC). During the reporting period, two IAEC meetings were conducted, ie., on 13th February and 5th November 2014. Progress of seven ongoing projects was reviewed by the committee and two new projects were approved for use of animals for experiments. Health status of the animals is being monitored by a visiting veterinary doctor.

3.2.3 *Brugia malayi* (sub-periodic) filarial parasite colony

The sub-periodic strain of *Brugia malayi* filarial parasite is maintained in two animal models, viz., mongolian gerbil (*Meriones unguiculatus*) and multimammate rat (*Mastomys coucha)*. Mosquito stages of the parasite (L1 to L3) are developed in *Aedes aegypti* (Liverpool strain) by feeding them with microfilaraemic blood and the infective larvae (L3) obtained are inoculated to the animal models for development and patent infection. As on date, there are 15 multimammate rats and 5 mongolian gerbils harbouring the filarial parasite. Adults and mf collected from these animals are being used for 6 projects involving immunological and drug development studies.

3.2.4 Rearing and colonization of mosquitoes

Cyclic colonies of the following four species of mosquitoes are being maintained in the Rearing and Colonization laboratory.

Immature and adult mosquito specimens were supplied to various divisions/laboratories of the Centre for carrying out basic studies on biology, conducting bioassays and evaluating newer vector control tools/agents including bio-larvicides and diagnostics. In addition, mosquito specimens were also provided for conducting exhibition at Schools/Colleges/Primary Health Centres to create awareness among the students and public of vector borne diseases, mosquito life stages and their control.

3.2.5 Mosquitoes (*Diptera: Culicidae*)

- *Culex quinquefasciatus*
- *Anopheles stephensi*
- *Aedes aegypti*
- *Toxorhynchites splendens*

The following species of larvivorous fishes are also being maintained in the laboratory.

- *Gambusia affinis*
- *Poecilia reticulata*

Supplies from the Rearing & Colonization laboratory to other laboratories of VCRC and outside the Centre are given in **Table 3.8**

TABLE 3.8 Supplies from Rearing & Colonization Division to different laboratories

Species	Internal (within VCRC)					External*	Total
	Vector Biology & Control	Microbiology, Immunology & Bioinformatics	Human Resource Development	Chemistry	Vector Ecology & Surveillance	Kirumam-pakkam PHC, Puducherry	
Culex quinquefasciatus							
Immature stages	500	190250	63050	4200	–	450	258450
Adults	–	3225	1100	–	–	300	4625
Anopheles stephensi							
Immature stages	–	74200	2600	4200	–	450	81450
Adults	66350	800	3550	–	–	300	71000
Aedes aegypti							
Immature stages	–	34900	3150	4200	–	450	42700
Adults	–	400	1200	14150	1800	300	17850
Toxorhynchites splendens							
Immature stages	–	–	–	–	–	100	100
Adults	–	–	–	–	–	50	50

**Supplies were provided for Exhibition purpose*

UNIT

4

Publications

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UNIT

5

Meetings/Seminars/Symposia/ Confernces/Workshops/ Guest Lectures Delivered

Date	Particulars	Scientist
10–11 Jan	7th National Conference on Students' Medical Research, 2014 organized by Department of Community Medicine, Medical College, Thiruvananthapuram, Kerala	Dr. P. Jambulingam Dr. N. Pradeep Kumar
16–17 Jan	Task Force Review meeting on Malaria at NVBDCP Conference Hall, Bhubaneswar, Odisha organized by the State Health Department, Odisha	Dr. S.S. Sahu
20 Jan	JIPMER institutional Bio-safety committee meeting as an external expert at JIPMER	Dr. L.K. Das
30 Jan	Joint Monitoring Mission - Peer Review Meeting, held at NVBDCP, Delhi	Dr. P. Jambulingam
7 Feb	Institutional Animal Ethics Committee meeting of Pondicherry University	Dr. K.P. Paily
10 Feb	Southern Regional Official Language Conference at Vigyan Auditorium of the Structural Engineering Research Centre (CSIR) CSIR campus, Taramani, Chennai	Dr. L.K. Das
10 Feb	Participated and presented the progress and observations on laboratory and entomological surveillance and vector control in respect of vector borne diseases prevalent in Kerala during the meeting held at the Conference Hall of DHS Office, Kerala	Dr. N. Pradeep Kumar
18 Feb	56th meeting on Town Official Language meeting at JIPMER, Puducherry	Dr. L.K. Das
21–23 Feb	International Conference on Entomology, Punjabi University, Patiala, Punjab	Dr. S. Poopathi
22–23 Feb	CME Annual Conference on behalf of Indian Association of Medical Microbiologists conducted by the Dept. of Microbiology, Kasturba Medical College, Manipal	Dr. P. Jambulingam
25 Feb	Inter-sectoral Co-ordination Meeting for Monsoon preparedness to the outbreak of vector-borne diseases at the Chamber of District collector, Kottayam, Kerala	Dr. N. Pradeep Kumar
25–26 Feb	International Conference of NTD organized by Amrita Institute of Medical Sciences (AIMS), and Global Network of Neglected Tropical Diseases, held at Amritha Medical College, Kochi	Dr. P. Jambulingam
28 Feb	Scientific Advisory Group (SAG) meeting of Medicinal Plant Unit of ICMR as an expert member at ICMR headquarters, New Delhi	Dr. L.K. Das
1–10 Mar	Joint Monitoring Mission for Vector Borne Disease Programme in India organized by Ministry of Health & Family Welfare, India	Dr. P. Jambulingam
2 Mar	JMM Review meeting on Malaria at NVBDCP Conference Hall, Bhubaneswar, Odisha organized by the State Health Department, Odisha	Dr. S.S. Sahu
3 Mar	Meeting on Inter-sectoral co-ordination chaired by the state Finance Minister, for Monsoon preparedness at the Chamber of District collector, Kottayam, Kerala	Dr. N. Pradeep Kumar
10 Mar	13th Task force meeting on Biotech product & process development, Anna University, Chennai	Dr. S. Poopathi

Date	Particulars	Scientist
10–11 Mar	UGC sponsored National Seminar on "Bioscience and Conservation" NABCON-14 organized by the AVC College (Autonomous), Mayiladudurai, Tamil Nadu - delivered a special lecture on "Current Status of Zoonotic Diseases in India"	Dr. K. Gunasekaran
11 Mar	District level malaria review meeting at CDMO conference Hall at Koraput under the chairmanship of the Collector and District Magistrate, Koraput	Dr. S.S. Sahu
20 Mar	UGC sponsored National Workshop on "Analytical Methods and Tools in Biological Research (AMaTiBR '14) organized by the Annamalai University, Chidambaram, Tamil Nadu – delivered keynote address	Dr. K. Gunasekaran
21 Mar	Biosafety committee meeting of Perunthalaivar Kamaraj Krishi Vigyan Kendra, Puducherry	Dr. K.P. Paily
27 Mar	Patent hearing meeting, patent office, Dwarka, New Delhi	Dr. S. Poopathi
28–29 Mar	Meeting of the three ICMR institutes (RMRC-B, VCRC, NIMR) held at RMRC, Bhubaneswar to develop a comprehensive action plan to assist Govt. of Odisha for the control of malaria	Dr. P. Jambulingam Dr. K. Gunasekaran
5 Apr	ICMR-Tribal Health Research Forum meeting at RMRC, Belgaum	Dr. K. Gunasekaran
5 Apr	District level malaria review meeting at CDMO conference Hall at Koraput under the chairmanship of the Collector and District Magistrate, Koraput	Dr. S.S. Sahu
7 Apr	Seminar organized by DMO, Alleppey in connection with the World Health Day – delivered a talk	Dr. N. Pradeep Kumar
7 Apr (FN)	World Health Day celebrated by the Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry – participated as a resource person and delivered a lecture on "Small bite big threat"	Dr. K. Gunasekaran
7 Apr (AN)	World Health Day celebrated by the National Vector Borne Disease Control Programme, Govt. of Puducherry – delivered a speech on "Vector Borne Diseases – Small creatures, Big threat with the Goal Better protection from Vector-Borne Diseases (VBD)"	Dr. K. Gunasekaran
7–8 Apr	Informal Expert Consultation on Vector Borne Diseases held at WHO-SEARO, Delhi	Dr. P. Jambulingam
10–11 Apr	18th Academic Council Meeting, Thiruvalluvar University, Vellore	Dr. S. Poopathi
15–16 Apr	Scientific Advisory Group Meeting of Division of ECD held at ICMR, Delhi	Dr. P. Jambulingam
15–16 Apr	Inter District Co-ordination Workshop 'for planning of malaria control activities with special reference to the vector control plan for ten cluster II districts' at Kakiriguma of Koraput district organized by the State Health Department, Odisha	Dr. S.S. Sahu
28–30 Apr	RTAG Meeting, WHO, SEARO, Delhi	Dr. P. Jambulingam
30 Apr	Vidyalaya Management Committee Meeting at Kendriya Vidyalaya, Koraput	Dr. S.S. Sahu
7 May	Evaluation of the progress of Ph.D. Scholar for CSIR JRF to SRF extension of third year at Department of Chemistry, Pondicherry University, Puducherry – as external expert member	Dr. Nisha Mathew
9 May	Review meeting on pre-monsoon preparedness at the NRHM Conference Hall of District Hospital, Kottayam, Kerala	Dr. N. Pradeep Kumar
29 May	Meeting on 'Finalization of SOP and Common Protocol at ICMR, New Delhi	Dr. K. Gunasekaran
31 May	Seminar on urban mosquito control, Life-science Intelligentsia, Chennai	Dr. S. Poopathi
7 Jun	Task Force cum Inter Sectoral co-ordination meeting on 'observation of Anti-Malaria Month (AMM) and Prevention of Vector borne and Water borne diseases' at O/o CDMO, Koraput, Odisha	Dr. S.S. Sahu
11 Jun	Meeting to discuss the action plan for implementation of Independent appraisal of LF Elimination Programme held at NVBDCP, Delhi	Dr. P. Jambulingam
16 Jun	Rapid Response Team meeting (Fever outbreaks and its containment) – District Collector & Health Officials, Kottayam, Kerala	Dr. N. Pradeep Kumar

Date	Particulars	Scientist
18 Jun	Task Force cum Inter Sectoral co-ordination meeting on Preventive measures of control of vector borne diseases at NAC Conference Hall, Jeypore, Odisha	Dr. S.S. Sahu
20 Jun	Meeting on Independent appraisal of lymphatic filariasis (LF) elimination programme in India held at NVBDCP, Delhi	Dr. P. Jambulingam
1–4 Jul	Independent appraisal of LF Elimination Programme in India – Gujarat: State level review meeting (Ahmadabad & Surat)	Dr. L.K. Das
5 Jul	Independent appraisal of LF Elimination Programme in India – Odisha: State level review meeting (Bhubaneswar)	Dr. K. Gunasekaran
18 Jul	Independent appraisal of LF Elimination Programme in India – Odisha: District level review meeting (Jagatsinghpur)	Dr. K. Gunasekaran
19 Jul	Task Force meeting on Malaria, Dengue and Diarrhea (MDD) preparedness meeting at O/o CDMO, Koraput, Odisha organized by the District Health Department, Koraput	Dr. S.S. Sahu
28–30 Jul	Independent appraisal of LF Elimination Programme in India – Gujarat: Evaluation of the progress	Dr. L.K. Das
30 Jul	Doctoral Committee meeting at JIPMER, Puducherry	Dr. K.P. Paily
21–28 Aug	Independent appraisal of LF Elimination Programme in India – West Bengal: State & district level review meeting & evaluation of the progress	Dr. P. Jambulingam
21 Aug	JIPMER institutional bio-safety committee meeting	Dr. L.K. Das
22 Aug	Malaria Group Meeting at ICMR, New Delhi	Dr. L.K. Das
28 Aug	Doctoral committee meeting, Pharmacology Department, JIPMER, Puducherry	Dr. Nisha Mathew
1 Sep	Seminar on Importance of taxonomy in conservation of faunal diversity held at Zoological Survey of India, Chennai - delivered a talk on "Diversity of VBD and Biodiversity"	Dr. P. Jambulingam
9–10 Sep	Meeting of Global collaboration for development of pesticides for public health (GCDPP) held at WHO, Geneva, Switzerland	Dr. P. Jambulingam
11 Sep	Delivered a lecture on 'Control of Dengue' held at O/o CDMO, Koraput, Odisha	Dr. S.S. Sahu
12 Sep	Meeting to discuss the action plan for achieving the goal of LF elimination held at Ministry of H&FW, New Delhi	Dr. P. Jambulingam
17 Sep	Workshop on Transmission Assessment Survey (TAS) in connection with ELF Programme in Koraput, Odisha	Dr. K. Gunasekaran
17 Sep	Meeting for planning of IVM for the control of cutaneous leishmaniasis in Thiruvananthapuram District held at DHS, Kerala	Dr. N. Pradeep Kumar
22 Sep	Meeting for planning the insecticidal spray operations in the tribal villages held at O/o DMO Trivandrum	Dr. N. Pradeep Kumar
30 Sep	Special lecture on control of diseases caused by mosquitoes – Focus on hygiene related issues at Idhaya College of Arts and Sciences for Women, Puducherry	Dr. K. Gunasekaran
10–12 Oct	X Joint Annual Conference of ISMOCD & IAE, Goa	Dr. S. Poopathi
16–18 Oct	25th National Congress of Parasitology on "Global Challenges in the Management of Parasitic Diseases" held at CSIR-CDRI Lucknow - oral presentation on "DNA based Electrochemical Biosensor for the detection of filarial parasite *Wuchereria bancrofti* in the vector"	Dr. V. Vasuki
21 Oct	Working Group Meeting on the Effective Use of Space Technology Tools held at ICMR, New Delhi	Dr. S. Subramanian
3 Nov	Seminar on "Functional genomics applications for developing methods to control pests and disease vectors" at Pondichery University, Puducherry	Dr. V. Vasuki
16 Dec	ICMR University Status - DPR Evaluation Meeting held at National Institute of Pathology, Delhi	Dr. P. Jambulingam
18 Dec	Insecticide Expert Group meeting organized by National Centre for Disease Control at Delhi	Dr. K. Gunasekaran

5.2 International Women's Day Celebration

International Women's Day was celebrated on 7th March 2014 and all the staff members participated in the celebration. Kalaimamani Smt. Meenakshi Devi Bhavanani, a professional journalist and teacher of Bharat Natyam and Ashtanga Yoga, was the Chief Guest of the occasion. On her behalf Dr. Meena Ramanathan, Yoga Teacher, delivered a talk on "How yoga empowers women" by highlighting the role of yoga in establishing a spiritual, moral, physical and cultural stability in every human being particularly in womenfolk. She also pointed out that uplifting women will elevate not only the society but promote a stronger country as a whole. During the celebration, in door games and singing competitions were conducted for the women students and staff of VCRC and prizes distributed. As a follow up activity of last year's resolution, all the staff of our Centre contributed voluntarily a sum of Rs. 10,915/- and was handed over to Hemophiliac Society, Puducherry Chapter, for treating poor hemophiliac students and patients. Osteoporosis is a common complaint in aging women and hence Bone Density Testing for the women staff of VCRC was arranged at JIPMER. "Healthy women make stronger India" is the message of this year.

5.3 Official Language Implementation

राजभाषा (हिन्दी) वार्षिक रिपोर्ट

आई. सी. एम. आर (ICMR) महानिदेशक की अपील पर, केंद्र मे दो दिनों दिनांक 25/9/2014 और 26/9/2014 पर हिन्दी दिवस सफलतापूर्वक मनाया गया था । केंद्र की कर्मचारियों और छात्रों के बीच विभिन्न प्रतियोगिताओं आयोजित की गई। गायन, अच्छा हस्तलिपी, सब्द अनुवाद और सब्द सक्ति प्रतियोगीता आयोजित की गई। प्रो. वि. विजयलक्ष्मी, मुख्य, हिन्दी विभाग, पोण्डिचेरी विश्वविद्यालय, पुधुच्चेरी, इस अवसर पर वे मुख्य अतिथि थे और पुरस्कार वितरण किये । इसके अलावा कर्मचारियों और छात्रों ने नगर राजभाषा कार्यान्वयन समिति (TOLIC), जीपमार (JIPMER) द्वारा आयोजित हिन्दी पखवाड़े उत्सव के अवसर पर आयोजित विभिन्न प्रतियोगिता में भाग लिये और पुरस्कार जीते।

ईस वर्ष हिंदी शिक्षण योजना में कर्मचारियों प्रशिक्षण लिये हैं। वर्ष के दौरान श्री दिनेश चन्द्र त्रिपाठी, सहायक निदेशक, हिन्दी विभाग, आई. सी. एम. आर मुख्यालय, नई दिल्ली से दौरा किये और केंद्र की द्वारा भारत सरकार की जारी राजभाषा नीति के कार्यान्वयन पर प्रयासों का आकलन और अनुपालन पर जायजा लिये,और बहुमूल्य सलहा दिये ।

इस साल मे चार त्रैमासिक राजभाषा कार्यान्वयन समिति (VCRC OLIC) की बैठक आयोजित की गई। तिमाही प्रगति रिपोर्ट आई. सी. एम. आर (ICMR) नई दिल्ली को भेजा गया था। नगर राजभाषा कार्यान्वयन समिति (TOLIC), जीपमार (JIPMER), पुडुचेरी में दो अर्ध - वार्षिक भेजा गया था। वर्ष के दौरान VCRC OLIC के अध्यक्ष और सदस्य सचिव जीपमार (JIPMER), पुधुच्चेरी में नगर राजभाषा कार्यान्वयन समिति और उप समिति बैठकों में भाग लिये ।

UNIT

6

Institutional Committees

Members of 36th SAC meeting of VCRC

Dr. A.P. Dash
Chairman
Former Regional Adviser (VBDC)
WHO-SEARO,
Flat No. 112 FF, Milano Mahagun Mansion II,
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Ghaziabad – 201 014, Uttar Pradesh

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Member
Director
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Member
Former Director, NIV
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Dr. P.L. Joshi
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Former Director, DRDE
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Regional Medical Research Centre,
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Statutory Member
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Statutory Member
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Statutory Member
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Regional Medical Research Centre for Tribals,
Nagpur Road,
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Dr. Pradeep Das
Statutory Member
Director
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Agamkuan, Patna – 800 007

Dr. G.S. Toteja
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Director
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New Pali Road,
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Statutory Member
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Institutional Human Ethics Committee (IHEC) Members

Prof. C. Adithan
Chairman
Sr. Professor & Head
Department of Clinical Pharmacology,
JIPMER,
Puducherry – 605 006

Dr. V. Govindaraj
Member
Medical Superintendent (Retd.)
Indira Gandhi Govt. Medical College &
Research Institute,
Kadirkamam,
Puducherry – 605 009

Dr. V. Balu
Member
Dean (Retd.)
Mother Theresa Institute of Health Sciences,
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Dr. S. Gunasekaran
Member
Dean (Retd.) of Humanities Studies
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Paruthipattu,
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Member
Prof. & Head, Dept. of Pharmacology
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Research Institute,
Kadirkamam,
Puducherry – 605 009

Dr. L.K. Das
Member Secretary
Scientist F
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

Institutional Animal Ethics Committee (IAEC) Members

Dr. P. Jambulingam
Chairman
Director
Vector Control Research Centre,
Indira Nagar,
Puducherry– 605 006

Dr. A. Yogamoorthi
CPCSEA main nominee
Reader,
Dept. of Ecology & Environmental Sciences,
Pondicherry University,
Puducherry – 605 014

Prof. S.C. Parija
CPCSEA link nominee
HOD of Microbiology,
JIPMER,
Puducherry – 605 006

Prof. V.N. Rao
Veterinarian
HOD of Veterinary Clinical Medicine (Retired)
RGCV & AS,
Puducherry – 605 006

Dr. B. Kumaran
Scientist from outside the institute
Associate Professor in Zoology
Kanchi Munivar College for Post Graduate Studies,
Lawspet, Puducherry – 605 008

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Non scientific socially aware member
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Biological scientist
Scientist G (Retd.), Consultant,
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Indira Nagar,
Puducherry – 605 006

Dr. A.M. Manonmani
Scientist from different discipline
Scientist F
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

Dr. K.P. Paily
Scientist in-charge of animals facility
Scientist F
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

Equipment Purchase Committee

Dr. B.N. Harish
Chairman
Professor of Microbiology
JIPMER, Puducherry – 605 006

Mr. Marie Stanislas Ashok
Member
Head
Computer Centre, Pondicherry University,
Puducherry – 605 014

Dr. A.M. Manonmani
Member
Scientist F
Vector Control Research Centre,
Puducherry – 605 006

Dr. R.L. De Britto
Member
Scientist F
Vector Control Research Centre,
Puducherry – 605 006

Dr. Nisha Mathew
Member
Scientist E
Vector Control Research Centre,
Puducherry – 605 006

Mrs. B. Parassacty
Member Secretary
Private Secretary
Vector Control Research Centre,
Puducherry – 605 006

Building Committee

Dr. K. Gunasekaran
Chairman
Scientist G
Vector Control Research Centre,
Puducherry – 605 006

Dr. K.P. Paily
Member
Scientist F
Vector Control Research Centre,
Puducherry – 605 006

Dr. A.R. Rajavel
Member
Scientist E
Vector Control Research Centre,
Puducherry – 605 006

Dr. V. Vasuki
Member
Scientist D
Vector Control Research Centre,
Puducherry – 605 006

Administrative Officer
Member
Vector Control Research Centre,
Puducherry – 605 006

Accounts Officer
Member
Vector Control Research Centre,
Puducherry – 605 006

Er. Kalyanasundram
External Member
Retd. Superintending Engineer
PWD, Govt. of Puducherry (Civil)

Er. N. Ayyadurai
External Member
Retd. Superintending Engineer
Electricity Department,
Govt. of Puducherry (Electrical)

Mr. K. Sundararajan
Member Secretary
Section Officer
Vector Control Research Centre,
Puducherry – 605 006

Hygiene Committee

Dr. P. Jambulingam	*Chairman*
Dr. S. Poopathi	*Working Chairman*
Dr. (Mrs.) B. Nandha	*Member*
Mrs. G. Jeyakodi	*Member*
Mr. R. Sathish Kumar	*Member*
Mr. B. Kumaresan	*Member*
Mr. S. Bhoopal Chakravarthi	*Member*
Mr. P. Arumugam	*Student Member*
Dr. R.L.J. De Britto	*Member Secretary*

Electronic File Management System Committee

Dr. K. Harikishan Raju	*Chairman*
Mrs. G. Jeyakodi	*Member*
Mrs. J. Kalaiselvi	*Member*
Mr. R. Sathish Kumar	*Member*
Mrs. B. Parassacty	*Member Secretary*

General Maintenance Committee

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. A.R. Rajavel	*Member*
Dr. V. Vasuki	*Member*
Mr. K. Sundararajan	*Member Secretary*

Condemnation Committee

Dr. K. Krishnamoorthy	*Chairman*
Dr. L.K. Das	*Member*
Dr. A.M. Manonmani	*Member*
Dr. R. Srinivasan	*Member*
Accounts Officer	*Member*
Mrs. B. Parassacty	*Member Secretary*

Environmental Safety Committee/ Biosafety Committee

Dr. L.K. Das	*Chairman*
Dr. A.M. Manonmani	*Member*
Dr. R.L.J. De Britto	*Member*
Dr. K.P. Paily	*Member*
Dr. C. Sadanandane	*Member*
Mr. K. Sundararajan	*Member Secretary*

Purchase Committee

Dr. K.P. Paily	*Chairman*
Dr. S. Subramanian	*Member*
Dr. Nisha Mathew	*Member*
Dr. A.R. Rajavel	*Member*
Dr. V. Vasuki	*Member*
Mrs. B. Parassacty	*Member Secretary*

Equipment Maintenance Committee

Dr. R.L.J. De Britto	*Chairman*
Dr. S. Subramanian	*Member*
Dr. Nisha Mathew	*Member*
Dr. V. Vasuki	*Member*
Mrs. B. Parassacty	*Member Secretary*

Official Language Implementation Committee

Dr. L.K. Das	*Chairman*
Mr. B. Kumareson	*Member*
Mr. P.M. Azad	*Member*
Mrs. N. Caliany	*Member*
Mr. Y. Srinivas Murty	*Member Secretary*

Grievance/Staff Welfare Committee

Dr. C. Sadanandane	*Chairman*
Dr. I. Geetha	*Member*
Mr. P.Kumaran	*Member*
Mr. K. Karunakaran	*Member*
Mr. T. Mohanan	*Member*
Mrs. T. Ahila	*Member Secretary*

Library Committee

Dr. A. M. Manonmani	*Chairman*
Dr. R.L.J. De Britto	*Chairman*
Dr. Nisha Mathew	*Member*
Dr. C. Sadanandane	*Member*
Sr. Library & Information Officer	*Member Secretary*

Vehicle Maintenance Committee

Dr. S. Subramanian	*Chairman*
Dr. A. R. Rajavel	*Member*
Mr. Joseph Suresh	*Member*
Mr. A. Elango	*Member*
Mr. R. S. Mariappan	*Member Secretary*

Committee for prevention of sexual harassment of women in work place

Dr. V. Vasuki	*Chairman*
Dr. R. Srinivasan	*Member*
Mrs. Vasumathi Nagarajan	*Member*
Mrs. B. Parassacty	*Member*
Dr. B. Nandha	*Member Secretary*

Management Committee

Dr. K. Krishnamoorthy	*Chairman*
Dr. K. Gunasekaran	*Co-chairman*
Dr. L.K. Das	*Member*
Dr. K.P. Paily	*Member*
Dr. A.R. Rajavel	*Member*
Dr. V. Vasuki	*Member*
Dr. C. Sadanandane	*Member*
Accounts Officer	*Member*
Administrative Officer	*Member Secretary*

UNIT

7

Staff Position

DIRECTOR

Dr. P. Jambulingam

Scientific

Dr. M. Kalyanasundaram*	*Scientist - G*
Dr. S. Sabesan*	*Scientist - G*
Dr. K. Krishnamoorthy**	*Scientist - G*
Dr. K. Gunasekaran	*Scientist - G*
Dr. Lalit Kumar Das	*Scientist - F*
Dr. (Mrs) A.M. Manonmani	*Scientist - F*
Dr. S. Poopathi	*Scientist - F*
Dr. R.L.J. De. Britto	*Scientist - F*
Dr. N. Pradeep Kumar	*Scientist - F*
Dr. K.P. Paily	*Scientist - F*
Dr. S. Subramanian	*Scientist - E*
Dr. (Mrs.) Nisha Mathew	*Scientist - E*
Dr. Sudhansu Sekar Sahu	*Scientist - E*
Dr. A.R. Rajavel	*Scientist - E*
Dr. (Mrs.) V. Vasuki	*Scientist - D*
Dr. R. Srinivasan	*Scientist - D*
Dr. C. Sadanandane	*Scientist - C*
Dr. (Mrs.) B. Nandha	*Scientist - B*

Library

Mrs. R. Sundrammal*	*Senior Library & Information Officer*

Administration & Accounts

Mr. R. Joseph Suresh*	*Administrative Officer*
Mrs. Vasumathi Nagarajan	*Accounts Officer*
Mr. K. Sundararajan	*Section Officer*
Mr. S. Balasubramanian	*Section Officer*
Mrs. B. Parassacty	*Private Secretary*
Mrs. T. Ahila	*Assistant*
Mr. V. Meganathan	*Assistant*
Mr. Vidjeacoumar S. Raymond	*Assistant*
Mrs. D. Indhumathy	*Assistant*
Mr. R. Janarthanan	*Assistant*
Mr. P.N. Ninan	*Assistant*
Mr. N. Suresh Kumar	*Assistant*
Mr. R. Sathiskumar	*Assistant*
Mrs. J. Kalaiselvi	*Personal Assistant*

Technical

Mr. A. Elango	*Technical Officer - B*
Mr. G. Jeeva	*Technical Officer - A*
Mr. V. Padmanabhan	*Technical Officer - A*
Dr. (Mrs) K. Athisaya Mary	*Technical Officer - A*
Dr. (Mrs) Ambilikumar	*Technical Officer - A*
Mrs. K.S. Snehalatha	*Technical Officer - A*
Dr. (Mrs.) A. Krishnakumari	*Technical Assistant (Research)*
Mrs. Abidha	*Technical Assistant (Research)*
Mr. T. Vijayakumar	*Technical Assistant (Research)*
Dr. R. Natarajan	*Technical Assistant (Research)*
Mr. G. Prabakaran	*Technical Assistant (Research)*
Dr. K.N. Vijayakumar	*Technical Assistant (Research)*
Dr. (Mrs.) I. Geetha	*Technical Assistant (Research)*
Dr. N. Sivagnaname	*Technical Assistant (Research)*
Mr. M. Palaniyandi	*Technical Assistant (Research)*
Dr. K. Harikishan Raju	*Technical Assistant (Research)*
Mrs. T. Sankari	*Technical Assistant (Research)*
Mr. S. Muthukumarvel	*Technical Assistant (Research)*
Mr. N. Krishnamoorthy	*Technical Assistant (Research)*
Mr. A. Mathivanan	*Technical Assistant (Research)*
Mr. K. Vaidyanathan*	*Technical Assistant*
Mr. S. Kandasamy	*Technical Assistant*
Mr. K. Vivekanandan	*Technical Assistant*
Mr. K. Mathivanan*	*Technical Assistant*
Mrs. Regnakumari Packrisamy	*Technical Assistant*
Mr. B. Edwin	*Technical Assistant*
Mr. Md. Mustafa Baig	*Technical Assistant*
Mrs. T. Sonia	*Technical Assistant*
Mrs. K.P. Amju	*Technical Assistant*
Mrs. T. Sumathy	*Technical Assistant*
Mrs. Sana Prasad Rao	*Technical Assistant*
Mr. S. Agatheeswaran	*Technical Assistant*
Mrs. G. Vijayalakshmi	*Staff Nurse*

** Retired from service on superannuation during the year*
*** Retired from service on superannuation and reemployed*