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VECTOR CONTROL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

PUDUCHERRY

Annual Report **2015**

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

VECTOR CONTROL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

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Annual Report

2015

The contents of this Annual Report should not be reviewed, abstracted or quoted without the written permission of the Director

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PREFACE

It gives me an immense pleasure to reflect on the overall research performance of the Centre during the year. Our key priority area of research continued to be on the issues related to end game of Global/National programme for elimination of lymphatic filariasis (ELF). In view of the reemergence of Scrub typhus and Kyasanur Forest Disease, the Centre has initiated building capacity in the field research on eco-biocoenosis of these diseases. The issues related to other important vector borne diseases viz., malaria and dengue are being addressed by our Field Stations located in Koraput, Odisha and Kottayam, Kerala respectively.

A rapid and cost-effective xenomonitoring protocol developed earlier is being validated in various settings. A miniaturized version of Electro Chemical-biosensor was successfully fabricated for the detection of filarial parasite DNA in vector mosquitoes and we are planning to adapt it to assessing human infection. In the light of the reports of triple resistance in *An. culicifacies,* the secondary vector of malaria to DDT, malathion and deltamethrin in the southern districts of Odisha, it is proposed to undertake studies towards developing resistance management strategies. Following the report of human cases of scrub typhus in Gorakhpur, studies have been initiated on prevalence of scrub typhus vectors/rodent hosts and the pathogen, *Orientia tsutsugamushi*.

Isolation and development of potential microbial/chemical mosquito control agents and by products continued to be one of our priority areas. A mosquitocidal bacteria, *B. amyloliquefaciens* (VCRC B483), isolated recently was found to exhibit anti-microbial potential against Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE) strains.

The technologies, 'Production of *Bacillus thuringiensis* var. *israelensis* (VCRC B17) (MTCC 5596), a mosquitocidal biopesticide' and 'Production of Cyclosporin A, an immunosuppressive agent by *Tolypocladium* sp. a mosquito active fungus', were selected for exhibition on the theme "Innovation in Medical Science & Technology" organized by the Indian Council of Medical Research at Rashtrapati Bhavan on 11 March, 2015.

M.Sc. Public Health Entomology course and Ph.D. programme were continuing with full strength, and one candidate has been awarded Doctoral degree during this year. Under our linkage programme with Georgetown University, Washington DC, and St. Olaf, Minnesota, USA, five students have carried out short term projects on vectors and vector borne diseases.

Resource crunch in terms of man power depletion has been a major problem for the last few years. During the year, there has been a marginal increase in the fund flow. With decentralisation, efforts are being made to fill-up the vacancies, yet the fulfilment of man power needs - scientific as well as technical remained a challenge. Fortunately, the Centre has received unstinted support from the retired Senior Scientists/Former Directors through their inputs in taking policy decisions, identifying the future research needs, and the involvement of some of them in major field programme. This has really helped to tide over the resource crunch due to shortage of experienced cadre Scientists. This was possible only with the support of the Secretary to the Government of India, Department of Health Research and Director General of ICMR and the Scientists of ECD division of ICMR, New Delhi.

The support extended by ICMR Task Force (VBDSF & THRF), National Vector Borne Disease Control Programme (NVBDCP), New Delhi, Department of Science and Technology (DST), Govt. of India, WHO, Geneva, WHO-SEARO and LF Support Centre-Bill & Melinda Gates Foundation for various research programmes was remarkable.

The Scientific Advisory Committee and other Institutional Committees of the Centre have contributed significantly to promotion of research in the centre. The Directorate of Health & Family Welfare, Govt. of Puducherry, Tamil Nadu, Karnataka, Kerala and Odisha have extended their support to field research activities of the Centre.

I am greatly indebted to all the scientists and staff of our Centre for their incredible support and cooperation for the achievements of the Centre.

Dr. P. Jambulingam
Director

प्रस्तावना

इस केंद्र के संपूर्ण अनुसंधान संबंधी वार्षिक निष्पादन को प्रस्तुत करते हुए मुझे अपार हर्ष है । हमारी प्रमुख प्राथमिकता वैश्विक और राष्ट्रीय कार्यक्रम के तहत लिंफेटिक फ़ाइलेरियासिस उन्मूलन के क्षेत्र मे होने वाले रिसर्च को जारी रखना है । स्क्रब टायफास और क्यासनुर जैसी जंगली बीमारियों के फिर से उभर आने के बारे में केंद्र ने इन बीमारियों के रिसर्च के क्षेत्र मे एको बायोकोएनोसिस पर क्षमता बढ़ाने की शुरूआत की। हमारे ओड़ीशा में फील्ड स्टेशन कोरापुट और केरल में कोट्टायम में रोगाणुवाहकों से पैदा होने वाली अन्य बीमारियों जैसे मलेरिया और डंगू आदि पर अनुसंधान एवं रोकथाम का काम चल ही रहा है ।

एक त्वरित और किफ़ायती जेनोमोनिटरिंग प्रोटोकाल जो पहले ही विकसित किया जा चुका है उसको कई सेटिंग में परखा जा रहा है । रोगाणुवाहक मच्छरों के अंदर फाइलेरिया पेरासाइट के डी एन ए का पता लगाने के लिए इलेक्ट्रो बायो सेंसर के सूक्ष्म रूप को सफलता पूर्वके तैयार किया गया। और अब हम इससे मानव पर होने वाले संक्रमण पर आजमाने की योजना बना रहे हैं । जैसी की रिपोर्ट हैं कि ओड़ीशा के दक्षिणी जिलों में दूसरे रोगाणु वाहक (अनोफेलेस कुलीसिफेसिस) मलेरिया में डीडीटी, मेलाथिओन और डेल्टामेथ्रिन तीनें के प्रति प्रतिरोधक क्षमता बढ़ी है, इसके लिए रेसिस्टेंस मैंनेजमेंट स्ट्रेटिजी पर अध्ययन का प्रस्ताव किया गया है । गोरखपुर से मिली स्क्रूब्स टायफास रोगाणु वाहक के मानव संक्रमण की रिपोर्टों पर स्क्रूब्स टायफास वेक्टर और रोडेंट होस्ट पेथोगन, *Orientia tsutsugamushi* पर अध्ययन शुरू किया गया ।

आइसोलेशन और डेव्लपमेंट ऑफ पोटेन्सियल माइक्रोबायल / केमिकल मोसकीटो कंट्रोल एजेंट के बाइप्रॉडक्ट हमारी प्राथमिकताओं में से एक है। A मोस्कीटोसिडल बैक्टीरिया *B. amyloliquefaciens* (VCRC B483), isolated को Methicillin रेसिस्टेंस स्टफ़ीलोकस औरेयस और वांकोमायसिन रेसिस्टेंट एंटेरिकोकस (वी आर ई) के प्रति प्रभावी एंटी माइक्रोबयाल का हाल ही में पता चला है। यह गर्व का विषय है कि भारतीय चिकित्सा अनुसंधान परिषद (आईसीएमआर) द्वारा राष्ट्रपति भवन में 11 मार्च 2015 को आयोजित "Innovation in Medical Science & Technology" विषय पर आधारित प्रदर्शिनी में Production of *Bacillus thuringiensis* var. *israelensis* (VCRC B17) (MTCC 5596), a mosquitocidal biopesticide' और Production of Cyclosporin A, an immunosuppressive agent by *Tolypocladium* sp. a mosquito active fungus" जैसी तकनीकों को चुना गया।

एम.एस सी. पब्लिक हेल्थ Entomology कोर्स पीएच. डी. कार्यक्रम सफलता पूर्वक चलाए जा रहे हैं ।एक शोधार्थी को इस वर्ष में Doctoral डिग्री का गौरव प्राप्त हुआ है । हमारे पाँच छात्रों ने लिंकेज कार्यक्रम के अंतर्गत जॉर्जटाउन यूनिवेर्सिटी, वाशिंगटन डी सी, और सेंट ओलफ, मिनेसोटा, यूएसए से vectors अँड vector borne diseases पर शॉर्ट टर्म प्रोजेक्ट पर काम किया ।

संसाधनो की कमी के तहत मैन पावर की कमी पिछले कुछ वर्षों से एक बड़ी समस्या बनी हुई है । वर्ष के दौरान फ़ंड मिलने में बहुत थोड़ी सी वृद्धि हुई है । विकेन्द्रीकरण के साथ खाली पदों को भरने के लिए प्रयास किए गए फिर भी मैन पावर साईंटिफ़िक और तकनीकी दोनों की कमी एक जुनौती के रूप में बरकरार है ।

सौभाग्य से केंद्र को कुछ सेवानिवृत्त वरिष्ठ वैज्ञानिकों / पूर्व निदेशकों का सहयोग मिलने से नीतियाँ बनाने और आगामी शोध कार्य की आवश्यकताओं को जानने में मदद मिली । उनमें से कुछ का मेजर फील्ड प्रोग्राम में भी योगदान रहा । इस से वास्तव में अनुभवी कैडर वैज्ञानिकों की कमी को पूरा करने में मदद मिली । यह सब सचिच महोदय, भारत सरकार, भारतीय चिकित्सा अनुसंधान परिषद (आईसीएमआर) के महानिदेशक महोदय और ई सी डी डिवीजन, (आईसीएमआर) नई दिल्ली के वैज्ञानिकों के समर्थन एवं सहयोग से ही संभव हो सका ।

आईसीएमआर टास्क फोर्स (VBDSF & THRF), नेशनल वेक्टर बोर्न डीजीस कंट्रोल प्रोग्राम NVBDCP), नई दिल्ली, विज्ञान एवं तकनीकी विभाग (भारत सरकार), डबल्यूएचओ (WHO जेनेवा), WHO-SEARO और एलएफ़ सपोर्ट सेंटर – बिल एंड मेलिंडा गेट्स फाउंडेशन द्वारा विभिन्न अनुसंधान कार्यक्रमें में जो सहयोग और समर्थन मिला वह उल्लेखनीय है ।

दि साइंटिफिक एड्वाइजरी कमेटी और केंद्र की दूसरी संस्थागत कमेटियों द्वारा रिसर्च के काम को लगातार जारी रखने में महत्वपूर्ण योगदान दिया गया है । स्वास्थ्य एवं परिवार कल्याण सेवा निदेशालय, पुदुच्चेरी, तमिलनाडु, कर्नाटक और ओड़िशा से भी क्षेत्रीय अनुसंधानके क्रिया कलापों में समर्थन और सहयोग मिला है ।

मैं सभी इस केंद्र के सभी वैज्ञानिकों और स्टाफ का हृदय से आभार व्यक्त करता हूँ जिनके अतुल्य सहयोग और समर्थन से इस केंद्र को उपलब्धियां और सफलता मिली है ।

डॉ. पी. जम्बुलिंगम
निदेशक

EXECUTIVE SUMMARY

The important research activities and major achievements during 2015-2016 are given below:

Filariasis

Development of surveillance tools for Lymphatic Filariasis Elimination Programme

- A miniaturized version of Electro Chemical-biosensor was developed for the detection of filarial parasite DNA in vector mosquitoes for the first time. Further improvisation is under processing to make it a portable miniature detector.
- The two-stage cluster design based sampling strategy for collecting *Culex* vector mosquitoes and monitoring filarial infection in vector by PCR assay at sub-district level has been validated for its usefulness/feasibility in operational settings at an evaluation unit (district) level. The sampling protocol could be an alternative to transmission assessment survey (TAS) for post-MDA surveillance in an evaluation unit.
- Using the simulation model 'LYMFASIM', it was determined that to achieve elimination in high transmission settings, MDA must be continued at least for 12 years and levels of Mf and Ag-prevalence must be reduced to 0.1% and 2.0% respectively in children (6–7 years).

Morbidity Management and Disability Prevention

- Short-term observations on a cohort of lymphoedema patients revealed a perceptible improvement in skin colour (17.2%) and texture (20.8%) of the lymphoedema legs in patients practicing limb hygiene regularly, besides a reduction in intertrigo prevalence among 10% of the total cases observed.

Distribution of DEC fortified salt as a supplement to MDA for ELF

- Under a collaborative study with RMRC, Port Blair, distribution of DEC fortified salt has been initiated in Noncowry Islands of Andaman & Nicobar Islands, the lone focus of diurnally sub-periodic *W. bancrofti,* as a supplement to MDA towards the elimination of LF. Coverage and compliance rates are being monitored. Quality check of the medicated salt also is in progress.

Malaria/Leishmaniasis

Efficacy of long lasting insecticidal nets (LLINs) and resistance monitoring

- Information generated on the distribution and usage of long lasting insecticidal nets (LLINs) by NVBDCP in malaria endemic areas, facilitated the programme to plan net replacements to maintain universal coverage, besides the IEC activities for an effective and sustainable malaria control strategy.

Development of intervention model for malaria/leishmaniasis vector control

- In the comparative assessment of the impact of combo vector control LLINs, plus indoor residual spraying (IRS)] versus single measure (only LLIN or IRS) on malaria transmission in Koraput district of Odisha State, it has been shown that there was no significant difference between the three arms over the study period of two years.
- The recently concluded study in collaboration with the Directorate of Health Services (DHS), Government of Kerala in Thiruvananthapuram district, showed active transmission of cutaneous leishmaniasis infection and its potential risk of spreading among the Kani tribes. Steps have been taken collectively with the Department of Forest and Wildlife, Govt. of Kerala to initiate appropriate intervention measures.

Scrub Typhus

- Studies were initiated on the prevalence of scrub typhus vectors/rodent hosts and the pathogen, *Orientia tsutsugamushi* in areas reported for human cases in Gorakhpur. The overall chigger index was 13.8, which was well above the critical level of chigger index (0.69 per rodent) indicating the receptivity of the area. One of the shrew mice (*Suncus murinus*) was positive for Weil Felix test as well as by PCR assay, indicative of the presence of reservoirs and potential for transmission to human.

Vector Control Products

Isolation and development of newer microbial/chemical agents for vector control

- *Bacillus cereus* (VCRC-B540) isolated for the first time from the gut contents of marine fish (*Lutjanus sanguineus*) showed activities against mosquito larvae. However, safety aspects of the strain have to be studied before developing this strain further.

Anti-microbial activity of an indigenous mosquitocidal bacterium, *B. amyloliquefaciens*

- Crude metabolite(s) obtained from *B. amyloliquefaciens* (VCRC B483), were found to be effective against Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE) strains, indicating the anti-microbial potential of this mosquitocidal bacterium.

Development of alternate cost effective raw materials for *Bti* (VCRC B17) production

- Among thirteen nutrient sources tried for media optimization of *Bacillus thuringiensis* var. *israelensis* (VCRC B-17), horse gram and Bengal gram based production media proved to be 20 times cheaper than the conventional medium. The mosquitocidal toxins produced in these media exhibited 1.2 times higher activity than that seen with the toxins from conventional medium. Hence, these locally and easily available raw materials will serve as a cost effective medium for the production of mosquitocidal toxins from *Bacillus thuringiensis* var. *israelensis* (VCRC B-17), a biopesticide, which is expected to be available for mosquito control programmes shortly.

Patents granted/filed

- Process patent on 'Fly ash based mosquito larvicidal formulations of *Bacillus thuringiensis* var. *israelensis*' filed in Bhutan, Myanmar and Nepal.

Technology Transfer

- The technology for the production of the mosquito larvicidal formulation from *Bacillus thuringiensis* var. *israelensis* has been licensed to one more commercial firm i.e., the 12th firm.

Participation of VCRC in "Innovation in Medical Science & Technology" at Rashtrapati Bhavan

- Two technologies of the institute, '*Bacillus thuringiensis* var. *israelensis* (VCRC B17) (MTCC 5596), a mosquitocidal biopesticide' and 'Production of Cyclosporin A, an immunosuppressive agent by *Tolypocladium* sp. a mosquito active fungus', were selected for the exhibition on the theme "Innovation in Medical Science & Technology" organized by the Indian Council of Medical Research at Rashtrapati Bhavan on 11 March, 2015, as a part of week long "Festival of innovation".

Development of naphthoquinone analogues as macrofilaricidal agents

- Studies on in-vitro ADME properties of 11 promising macrofilaricidal compounds in comparison with standard drugs have been completed. Based on the ADME results, six compounds TR-NPQ 1, 2, 4-7 have been short listed for in-vivo screening for macrofilaricidal activity.

Supply of insecticide impregnated papers (IIPs) to National Vector Borne Disease Control Programme (NVBDCP) for monitoring susceptibility/resistance in vector mosquitoes in the country

- Standardization of the preparation of insecticide impregnated papers for monitoring vector resistance/susceptibility to insecticides has been completed. Quality checking was done in comparison with WHO papers. Validation of the insecticide impregnated papers were carried out independently at NIMR, Delhi and Institute of Vector Control and Zoonoses, Hosur.

Biomedical Informatics

'VectorInfo' a web repository of medically important Indian arthropods

- A web resource for biological aspects of each Indian vector species, genomic data resource for user screening and transcriptomic data resource including promoter region and promoters is designed.

Role of kdr mutations in voltage gated sodium channel (VGSC) in conferring target mediated resistance to DDT and synthetic pyrethroids in malaria vectors

- Three mutant models of VGSC such as L1014F, L1014H and L1014S were constructed. The mutant models were subjected to molecular dynamics simulations and the conformational variations were analysed. It is concluded that the L1014H and L1014S mutations drastically affected the structure of VGSC which could reflect in the activities of the channel also. These conformational variations in the sodium transport domain will affect the passage of the ions and molecules such as DDT and some other insecticides and inhibit them to enter into the cell thereby causing the insecticide resistance.

Studies to detect and identify antigenic determinants of proteins of *Wuchereria bancrofti*

- Four potential antigenic determinants (CCEP1, CCEP2, CCEP3 and CCEP4) on cuticular collagen (CC) protein which are unique to *Wuchereria bancrofti* were identified. Evaluation of the immunogenicity of each peptide is in progress.

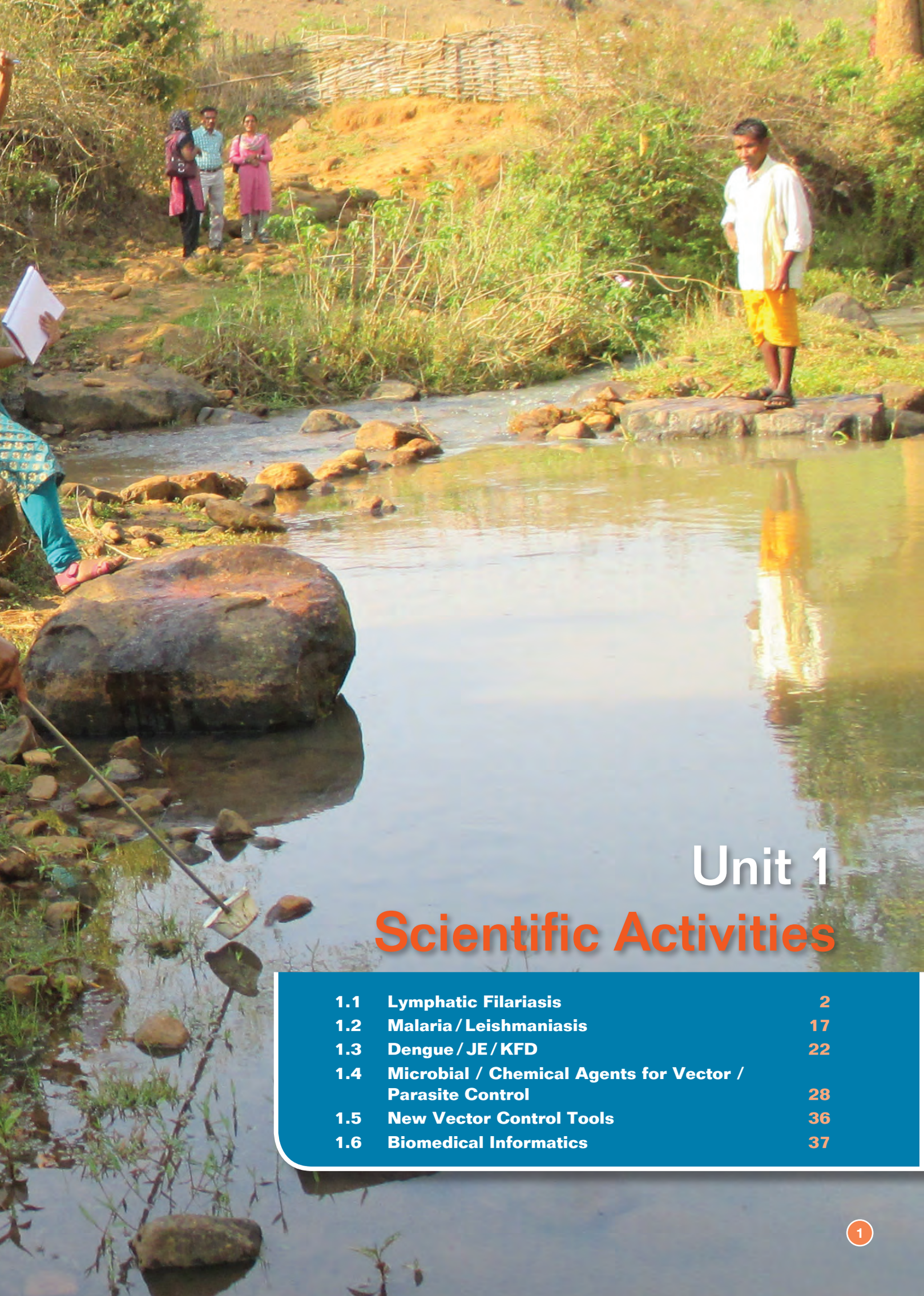
Human Resource Development

Academic

- **M.Sc. Public Health Entomology:** Eleven candidates have been admitted for the year 2014–16 (fifth batch) of M.Sc. Public Health Entomology course affiliated to Pondicherry University.
- **Ph.D. Programme:** Ten (Zoology – 7; Microbiology – 2; Chemistry – 1) candidates continue to pursue their Ph.D. programme. One candidate has been awarded Doctorate degree in Chemistry during 2015.

Training

- Three Medical Officers from Central Hygienic & Anti Epidemic Institute, Ministry of Public Health, DPR Korea, were given 4 weeks training on Malaria Entomology & Vector Control.
- Three students from Department of International Health, Georgetown University, Washington D.C, USA and two students from St. Olaf College, Minnesota, USA undertook their dissertation projects [6–15 weeks].
- 20 MD students from various Medical colleges have undergone observational training on the control of vectors and vector borne diseases.



Unit 1
Scientific Activities

1.1 LYMPHATIC FILARIASIS

1.1.1. 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, Senthil Kumar A (VIT University, Vellore)

A Prototype version of electro chemical biosensor developed earlier was further refined through improved asymmetric PCR for probe designing and standardization of chemically modified electrode (CME) and a signal detector (Annual Report, 2014).

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.

The final product was converted into a miniaturized, EC biosensor and a DNA probe sensitized CME for the specific detection of filarial parasite, *W. bancrofti* DNA in vector mosquitoes.

Miniaturized version of EC-biosensor using screen printed electrodes was developed as a tool for detecting filarial parasite DNA in vector mosquitoes. During the reporting period, real samples were tested in six experiments using the device and it was found that impedance signals of the real samples were highly reproducible (Figure 1.1). The project is completed and final report submitted to the funding agency and preparation of papers for filing patent is in progress.

1.1.2. Comparative evaluation of a new test strip against the currently available ICT for the detection of filarial antigenemia in humans

EM 1405: Aug 2014 – Jan 2015

Jambulingam P, Krishnamoorthy K, Subramanian S

The national programme for lymphatic filariasis (LF) elimination is nearing its endgame in at least 180 of the 250 implementation units (IU). The decision to stop Mass Drug Administration (MDA) and subsequent monitoring during post-MDA until certification is based on the WHO recommended transmission assessment survey (TAS). Immuno chromatographic card test (ICT) is being used in TAS for detecting circulating filarial antigenemia in children. Although, the test is a valuable tool in the global programme to eliminate LF, it is less stable (shelf life of 3 months in ambient temperatures in the tropics), expensive and its reliability is a constraint by the narrow time window (10 minutes) for reading test result: false positive results are common if the tests are read later than 20 minutes. Recently, a new test strip has been developed, validated and showed improved sensitivity and specificity elsewhere. Compared to ICT, the new test namely Filariasis Strip Test (FST) is reported to have a longer shelf life and be cheaper. However, data needs to be generated under Indian situation to assess its diagnostic ability and to arrive at a critical threshold for stopping MDA before being recommended for use in TAS in the national programme. This study aims at comparing the stability, sensitivity and specificity of the test strip against ICT under laboratory conditions and making a comparative assessment of the new test strip against the currently available ICT for TAS.

Objectives:

- To compare the stability, sensitivity and specificity of the test strip against ICT under laboratory conditions.
- To make a comparative assessment of the new test strip against the currently available ICT for TAS.

Laboratory evaluation: A total of 217 samples collected from antigen positive (Og4C3 based) cases were tested, using FST and ICT simultaneously. Blood sample was also collected from the same finger prick for Og4C3 assay. Results of FST and ICT were read at 10 minutes, 30 minutes and 24 hours. The test results were scored as "0" for negative reaction, 1+ (the test line is faint and weaker than control), 2+ (the test line is as strong as the control) and 3+ (the test line is stronger than control).

There were 92 positives detected by FST (42.4%) and 55 positives by ICT (25.3 %) at 10 minutes. The FST detected 67.3% more positives at 10 minutes. The sensitivity of FST was 100% and specificity was 77.2% when compared to ICT (Table 1.1). Reading at 30 minutes showed 47.9% positivity by FST and

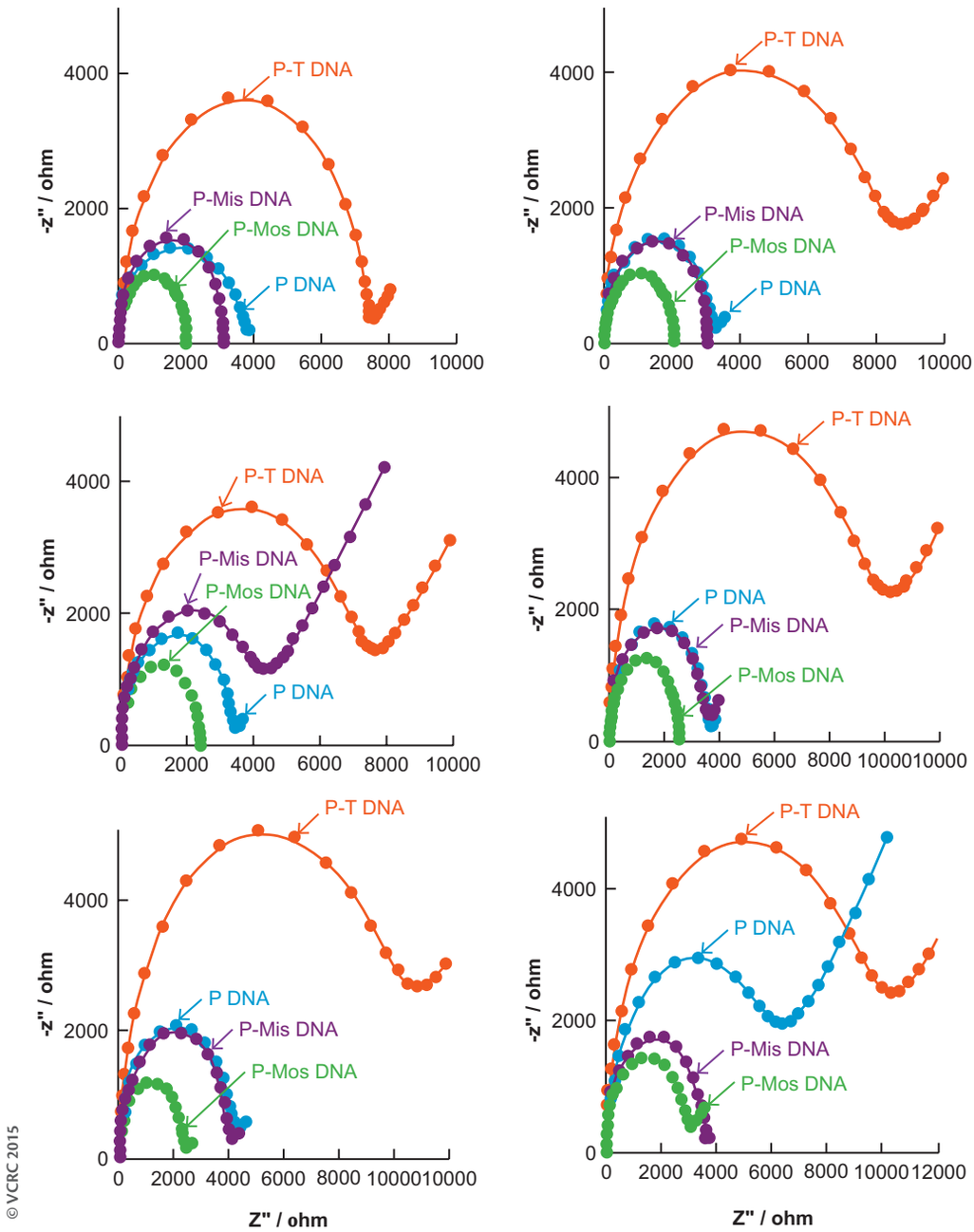


Figure 1.1 Nyquist plots of $Ru(bpy)_3^{2+}$ in 0.1M PBS obtained at various modified electrode with real samples; (a) GCE/GO+Chit/p-DNA, (b) GCE/GO+Chit/p-T DNA, (c) GCE/GO+Chit/p-Mos DNA and (d) GCE/GO+Chit/p-Mis DNA; (The data was reproduced for six times).

Table 1.1 Comparison of results of FST with ICT for samples read at 10 minutes

Test	ICT		
New strip test	**+ve**	**−ve**	**Total**
+ve	55	37	92
−ve	0	125	125
Total	**55**	**162**	**217**
Sensitivity (true positive)			100.0%
Specificity (true negative)			77.2%
False negative			0.0%
False positive			22.8%
Predictive value of a positive test			59.8%
Predictive value of a negative test			100.0%

29.1% by ICT. FST detected 5.7% more positives and ICT detected 3.8% more positives compared to reading at 10 minutes. Reading at 24 hours showed 58.1% positivity by FST and 40.1% by ICT. FST detected 15.7% more positives and ICT detected 14.8% more positives compared to reading at 10 minutes.

As per the test scoring, there were 54 positives with weak (1+), 28 with strong (2+) and 10 with stronger (3+) test reactions by FST at 10 minutes. The corresponding positives by ICT were 42, 11 and 2. The agreement was 100 percent with 3+, 96.4% with 2+ and 33.3% with 1+. Agreement differed with test results (Table 1.2).

Table 1.2 Comparison of test scores in the laboratory study with the ICT and the FST for samples read at 10 minutes

Test	ICT				
New strip test	0	1+	2+	3+	Total
0	125	0	0	0	125
1+	36	18	0	0	54
2+	1	22	5	0	28
3+	0	2	6	2	10
Total	55	42	11	2	217

Negative reactions (stability):

- 15 (12.0%) out of 125 negatives at 10 minutes by FST became positive (1+) by 30 minutes. Nine (5.6%) out of 162 negatives by ICT showed positive (1+) by 30 minutes.
- 37 (29.6%) out of 125 negatives at 10 minutes by FST and 34 (20.9%) out of 130 negatives by ICT showed positive by 24 hours.

Field evaluation: Comparative assessment of FST against ICT was carried out in two evaluation units (EUs) which are endemic and covered under MDA. This field evaluation was conducted in one district that was eligible for TAS and another where Mf prevalence in one of the sentinel sites was above 1% and MDA was being continued. School based TAS was conducted in both the EUs as the enrolment of children in the school was above 75%.

EU1: Trissur district in Kerala state was eligible for TAS and there were 367 schools with primary sections (Grade I and II) and the total numbers of students in the first and second grades were 32689. The output of Survey Sample Builder with an estimated non-response rate of 8% showed 1556 as the sample from 30 clusters (schools) and 18 as the critical cut-off.

The survey results are shown in Table 1.3. The results showed five antigen (Ag) positive by ICT and 24 by FST and FST detected 19 more positives. Two positive reactions by ICT showed negative reaction by FST. The sensitivity was 60.0% and the specificity was 98.6%. The Ag prevalence by ICT was estimated to be 0.32% while it was 1.54% by FST. All the five clusters with Ag positive children detected by ICT had FST positives, but there were more positives by FST than that detected by ICT. There were 7 additional clusters with FST test positives alone. Prevalence of Ag detected by FST is significantly correlated with ICT (R=0.58; P<0.05). The results showed that the Ag positives were below the critical cut-off by ICT test while it showed above by FST.

Table 1.3 Antigen screening test by ICT and FST in Trissur (district eligible for TAS)

Test	ICT		
New strip test	+ve	–ve	Total
+ve	3	21	24
–ve	2*	1530	1532
Total	5	1551	1556
*Tests will be repeated for reconfirmation			
Sensitivity (true positive)			60.0%
Specificity (true negative)			98.6%
False negative			40.0%
False positive			1.4%
Predictive value of a positive test			12.5%
Predictive value of a negative test			99.9%

Table 1.4 Antigen screening test by ICT and FST in Cuddalore (district not eligible for TAS)

Test	ICT		
New strip test	+ve	–ve	Total
+ve	4	4	8
–ve	0	1548	1548
Total	4	1552	1556
Sensitivity (true positive)			100.0%
Specificity (true negative)			99.7%
False negative			0.0%
False positive			0.3%

EU2: Cuddalore district in Tamil Nadu recorded >1% Mf prevalence in one of the sentinel sites and MDA was continued. There were 887 schools with primary section with total of 36837 students in first and second grades in this evaluation unit. The sample size estimated using SSB with an estimated non-responders of 10% is 1556 from 42 clusters (schools). The critical cut-off is 18. The results of the screening are given in Table 1.4.

Out of 1556 children screened, 4 (0.26%) were found to be Ag positive by ICT and 8 (0.52%) by FST. All the four positives detected by ICT showed positive by FST and the agreement for positive reaction by FST is 100%. Sensitivity and specificity were near total. With two clusters with Ag positive children detected by both ICT and FST, FST detected two more clusters. The number of Ag positives detected by ICT as well as FST was below the critical cut-off and hence the evaluation unit is eligible for stopping MDA.

The new Filariasis Strip Test showed high rates of sensitivity and specificity and could detect about 67.3% more positives compared to ICT. The agreement between the tests was more than 99% with strong test lines, but with weak positives, the agreement was only 33%. The positive results were

stable. Test readings after 10 minutes showed 28.7% more positives by FST while it was 40% by ICT as the negative test results at 10 minutes turned to be positive afterwards. The higher levels of detectability by FST compared to ICT will have implications in taking decision for continuing/ stopping MDA in the EUs.

1.1.3. 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, Krishna Kumari A

The study is being carried out in 31 villages around Dindivanam and Ginji of Villupuram district, Tamil Nadu with the following objectives.

Objectives:

- To assess the impact of ongoing MMDP programme on prevention of disability in the study area.
- To identify impediments for sustainability of ongoing MMDP programme.
- To identify suitable parameters for evaluation of the impact of MMDP Programme.

A total of 546 filarial lymphoedema patients were enumerated in 31 villages of Dindivanam and Gingee taluks of Villupuram district. The patients were categorized into three groups based on the morbidity management services they receive: Group 1 received MMDP from VCRC 10 years back and currently from Tamilnadu State NVBDCP, group 2 is receiving MMDP from Tamilnadu State NVBDCP from the beginning and Group 3 was under NVBDCP, but currently receiving from VCRC.

From each of the above three groups, following inclusion and exclusion patients were recruited for the study. Baseline information collected from patients of group 3 and details were provided in the annual report 2014. Under routine MMDP programme (Group 1) lymphoedema patients receive one time training and limb hygiene kit and subsequently report to peripheral health workers to get treatment for acute episodes from the nearest PHC. Under VCRC, one time training on limb hygiene was provided, limb hygiene kits were issued on monthly basis, and active surveys were carried out for detecting and treating Adeno Lymphangitis (ADLA).

In areas currently under VCRC training, limb hygiene was given at intake and patients are visited on monthly basis to collect the details of compliance of limb hygiene procedures in the prescribed format. In total, 60 patients (66 Lymphoedema (LE) legs), 62 patients (62 LE legs) and 64 patients (68 LE legs) were recruited from each group and three sets of procedures were completed at intake: KAP questionnaire, HRQoL questionnaire and medical officer's examination. The demographic and basic clinical information are provided in Table 1.5.

HRQoL assessment: One-time assessment at intake is completed. The data shows that in the current MMDP programme which has been in operation for more than a decade, the HRQoL scores are much below the maximum normal score of 144. Patients with higher grades of lymphoedema (grade-4) have scores 84.5% below normal score in Group 1, 73.9% below normal score in Group 2 and 75.9% below normal score in Group 3. In all the 3 groups the quality score for Lymphoedema grade 1 was highest and lymphoedema grade 4 was lowest. This shows that the HRQoL decreases with the progression of the disease.

The first follow up was completed in group 3 (current VCRC MMDP) after six months of intake. Table 1.6 depicts the changes in clinical parameters at six months follow up. It was observed that there are perceptible improvements in colour and texture of the lymphoedema legs in group 3

Table 1.5 Demographic and basic clinical details of the recruited LE patients

Details	Group 1		Group 2		Group 3	
Age group	**n**	**%**	**n**	**%**	**n**	**%**
31–45	13	21.7	9	14.5	13	20.3
45–60	27	45.0	34	54.8	32	50.0
60+	20	33.3	19	30.8	19	29.7
All	**60**	**100.0**	**62**	**100.0**	**64**	**100.0**
Gender						
Male	17	28.3	16	25.8	16	25.0
Female	43	71.7	46	74.2	48	75.0
All	**60**	**100.0**	**62**	**100.0**	**64**	**100.0**
LE Grade						
Gr1	2	3.0	2	3.2	0	0.0
Gr2	30	45.5	25	40.3	32	50.0
Gr3	20	30.3	27	43.5	23	35.9
Gr4	14	21.2	8	12.9	9	14.1
All	**66***	**100.0**	**62***	**100.0**	**64***	**100.0**
Intertrigo Present	**60**	**33.3**	**62**	**22.6**	**64**	**43.8**
LE Volume (ml)						
Upto–200	12	21.4	8	13.6	11	17.7
201–500	20	35.7	32	54.2	24	38.7
501–1500	16	28.6	14	23.7	20	32.3
>1500	8	14.3	5	8.5	7	11.3
All	**56**	**100.0**	**59**	**100.0**	**62**	**100.0**

*Lymphoedema legs

Table 1.6 Change in clinical parameters in Group 3 patients at 6 months follow-up

Clinical parameter	Site	Intake (N=66*)		I Follow-up (N=60*)	
		n	%	n	%
Color	Foot	31	46.9	27	45.0
	Leg-Lower 1/2	30	45.5	17	28.3
	Leg Upper 1/2	14	21.2	7	11.7
Texture	Foot	47	71.2	36	60.0
	Leg-Lower 1/2	50	75.8	33	55.0
	Leg Upper 1/2	34	51.5	27	45.0
Moisture	Foot	40	60.6	37	61.7
	Leg-Lower 1/2	30	45.5	18	30.0
	Leg Upper 1/2	15	22.7	6	10.0
Ulcer	Foot	7	10.6	9	15.0
	Leg-Lower 1/2	4	6.1	4	6.7
	Leg Upper 1/2	1	1.5	1	1.7
Wart	Foot	4	6.1	3	5.0
	Leg-Lower 1/2	2	3.0	2	3.3
	Leg Upper 1/2	1	1.5	1	1.7
Nodule	Foot	5	7.6	4	6.7
	Leg-Lower 1/2	5	7.6	3	5.0
	Leg Upper 1/2	2	3.0	1	1.7
Intertrigo	Present	28	42.4	19	31.7

patients. In addition, we observed more than 10% reduction in intertrigo prevalence at six months follow-up.

The baseline information on three groups has been constructed and six months follow up on group 3 is completed. It was observed that there were perceptible improvements in colour, texture and moisture of LE leg (Table 1.6). Six months follow up on group 2 and group 1 patients are being taken up between November 2015 and January 2016.

1.1.4. Prediction and evaluation of antigenic determinants of proteins of *Wuchereria bancrofti*

IM 1501: Feb 2015 – Jan 2017

Vasuki V & Nandakumar Y

Currently, Transmission Assessment Survey (TAS) is recommended for making decision on stopping MDA and Post-MDA surveillance until certification of elimination of LF. This is mainly based on detecting antigen in children (6–7 years) to verify absence of transmission. Post MDA monitoring until certification is based on ICT, which is imported and expensive. A new Filariasis Test Strip (FTS) has been evaluated and is yet to be put to use. An exploratory study was initiated with an aim of developing an indigenous antigen based diagnostic tool. 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. Cuticular collagen-encoding genes particularly Cuticular collagen 2 (*col* 2) have been described for filarial parasites and are highly immunogenic, making them potential therapeutic targets. We proposed to assess the antigenic determinants of the cuticular collagen 2 protein of *W. bancrofti* for their immunogenicity for the specific diagnosis of *W. bancrofti* infected individuals with an aim of developing an antigen assay.

Objectives:

- To predict antigenic determinants of the cuticular collagen 2 protein of *W. bancrofti* for synthesizing peptides.
- To evaluate the synthesized peptides for antigenicity and immunogenicity against human sera.

Based on the available sequence and structure of this protein, three epitopes of 37–72 aa were identified by using different prediction tools. The predicted epitopes were synthesized through outsourcing. Four peptides of 11–20 aa length were synthesized. The solubility of the peptides was determined in Triflouroacetic acid (TFA) and PBS (Phosphate buffered saline) in 1% solution. The peptides were soluble in TFA and PBS and immunogenicity of each peptide was tested at concentrations ranging from 0.1–5.0 µg against known mf positive and negative sera by ELISA. Peptides dissolved in PBS showed better immunoreactivity. Among the four peptides, peptide CCP2 and CCP4 showed higher immunogenicity at a concentration of 3.0 µg (Figure 1.2). Immunogenicity in terms of OD values differed between positive and negative human sera and were 1.38 (0.58) and 1.35 (0.75) respectively. However, the difference was inconsistent indicating lesser specificity of the assay.

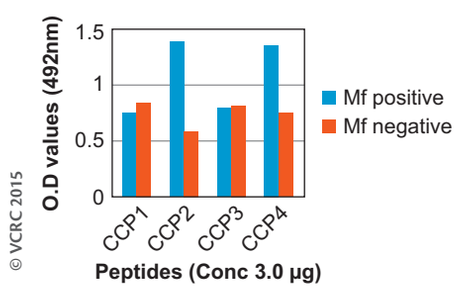


Figure 1.2 Immunoreactivity of four peptides against IgG antibody of *W. bancrofti* mf positive and mf negative human sera.

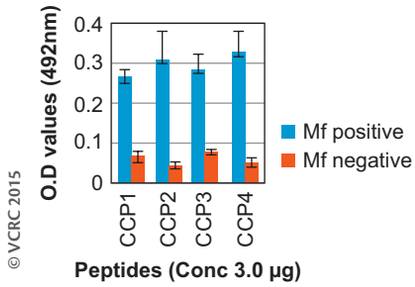


Figure 1.3 Immunoreactivity of four peptides against IgG4 antibody of *W. bancrofti* mf positive and mf negative human sera.

In order to increase the specificity of the assay, the secondary antibody-peroxidase labeled anti-human IgG (IgG4) was used in place of anti-human IgG (whole molecule) in the assay protocol. The immunogenicity obtained for CCP2 and CCP4 were 0.309 (0.04) and 0.327 (0.05) respectively showing improved specificity (Figure 1.3). Evaluation is in progress. Two peptide candidates, after confirming their immunogenicity, will be selected for monoclonal (anti-peptide) antibody production.

1.1.5. 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

In India, MDA has been stopped in at least 49 out of 255 IUs, since the number of children positive for Ag were below the critical level as per transmission assessment survey (TAS) protocol. 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 the potential to lead to resurgence and therefore warrants close monitoring during post-MDA period. 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. Besides surveillance, targeted interventions may be useful to liquidate the parasite load in the communities with antigen positive children in preventing 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. This study aims at addressing whether communities of antigen positive children detected by TAS require additional intervention during post-MDA.

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.

This study is carried out in Thanjavur district which received 14 rounds of MDA. Having achieved less than 1% microfilaraemia (Mf) prevalence in all the sentinel and spot-check sites as well as in additional 10 sites, this district was qualified for TAS to decide on stopping MDA. This district was divided into two evaluation units and TAS results showed infection below the critical cut-off and further intervention was stopped. However, two clusters viz., Vazhkai subcentre with a population of 5696 in Kabisthalam PHC (Arm 1) and Koodananal subcentre with a population of 7876 in Budalur PHC (Arm 2) were found to have antigen positive children. These two clusters are selected, one for administering two rounds of MDA at six monthly intervals and another as control (Annual Report 2014). Baseline data on Mf prevalence in the community and antigenemia prevalence in 6-7 years old children have been collected. Sample size for Mf-survey in each arm was calculated based on an expected prevalence of 1% with 95% confidence level and 0.3% precision. Accordingly, the estimated minimum sample size was ~2500 for Arm 1 and ~2800 for Arm 2.

Antigenemia prevalence among children was above the critical cut-off of 2% in three villages (Table 1.7). In Vazhkai subcentre, as many as 2333 individuals were screened for Mf. Persistence of infection was observed in four of the five villages. Examination of slides collected from 2054 individuals in Koodananal subcentre and Mf-survey from the remaining villages and vector survey are in progress.

Out of the two clusters, one arm will be selected for targeted MDA. Two rounds of MDA will be carried out at six monthly intervals in the intervention arm. Intensive social mobilization and advocacy will be carried out to maximize community compliance. Coverage of drug distribution and consumption will be assessed by questionnaire based sample survey. Vector infection will

Table 1.7 Antigenemia prevalence among children in selected villages

PHC	SUB centre	Village	Population	Mf sample	Mf +ve	Mf rate	Ag (6-7 years) sample	Ag +ve	Ag rate
Kabisthalam	Valkai	Valkai	1447	655	5	0.76	44	3	6.82
Kabisthalam	Valkai	Sathiyamangalam	1878	707	3	0.42	43	1	2.33
Kabisthalam	Valkai	Nakkampadi	458	245	0	0.00	9	0	0.00
Kabisthalam	Valkai	Koilpathu	1008	345	1	0.29	2	0	0.00
Kabisthalam	Valkai	Ramanujapuram	905	381	1	0.26	33	0	0.00
Valkai Total			**5696**	**2333**	**10**	**0.43**	**131**	**4**	**3.05**
Budalur	Koodananal	Koodananal	635*	190	0	0.00	0	0	0.00
Budalur	Koodananal	Onbathuveli	4617*	1418	2	0.14	102	4	3.92
Budalur	Koodananal	Thirukkattuppalli	5234*	446	0	0.00	31	0	0.00
Koodananal Total			**10486**	**2054**	**2**	**0.10**	**133**	**4**	**3.01**

*Observation in progress

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.

1.1.6. 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 – Sep 2016

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

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The national LF elimination programme is nearing its endgame in many of the endemic districts. As of 2015, 49 (66 evaluation units) of the 255 endemic districts have passed the transmission assessment survey (TAS) and MDA has been stopped in these districts. In all these districts, it is necessary to monitor infection either in human or vector. TAS is the recommended strategy to monitor infection in human during post-MDA period. Monitoring infection in vector ('xenomonitoring') through PCR assays could be a potential tool for post-MDA surveillance strategy besides an alternative to TAS.

As reported in 2014 Annual Report, the VCRC has developed and validated rapid and cost-effective surveillance tools (gravid trap for vector collection, PCR assay for detecting vector infection, and sampling strategies) in one of the PHCs in Thanjavur district, Tamil Nadu that could identify the low level of infection following multiple annual rounds of MDA. Further, results of simulated sample surveys 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. The present study is a continuation of our earlier work to assess whether the demonstrated surveillance tools are useful to assess the long term changes in vector infection after stopping MDA and to assess if sampling effort could be further reduced.

Objectives:

- To develop, and validate sampling strategies for monitoring vector infection by PCR as a surveillance tool for assessing post-MDA situation against antigen based TAS.
- To assess the long term changes in vector infection after stopping MDA.

Ammapettai PHC, in Thanjavur district is the study area. The PHC had undergone eight rounds of MDA by 2010 resulting in an Mf-prevalence of 0.2%. Our earlier study results showed a decline in vector infection when the mosquito survey was repeated two years after stopping MDA. The results also indicated that the vector infection was comparable with human infection and hence can also be an alternative to TAS based on human blood sampling in the post-MDA surveillance period (Annual Report 2014).

The extended study was carried out in two steps: (1) vector sampling in the entire PHC (PHC area), and

(2) vector sampling in 'hot-spots' (hotspot area) in the same PHC. In step 1, a total of 165 households (HH), spread over 20 villages and 15 wards in the entire PHC were selected following a systematic sampling protocol. In step 2, following the same sampling protocol, a total of 153 HHs spread over 17 streets in four hotspots were selected. Mosquito sampling was done by fixing gravid traps in the selected households. In each step, the surveys were repeated twice at an interval of one month. The two surveys in step 1 were planned to assess consistency of the estimates between surveys and in step 2 provide an indication of its usefulness as a complement to step 1.

In the PHC area, a total of 11200 and 11228 *Culex* females were collected using 485 and 629 trap nights in surveys 1 and 2 respectively. In the hotspot area, a total of 11746 and 13006 *Culex* females were collected using 312 and 387 trap nights respectively in surveys 1 and 2 (Figure 1.4). The per trap density do not differ significantly between surveys 1 and 2 in both PHC (Survey 1 vs 2: 23.1 vs 17.9) and hotspot (Survey 1 vs 2: 37.6 vs 33.6) areas (P>0.05 for both comparisons). The density (combined for both the surveys) was significantly higher in the hotspot area than that in the PHC area (P<0.05), suggesting that the hotspot areas are highly receptive. In all the surveys gravid females ranged between 83 and 92%.

A total of 10044 (60.8%) and 9628 (58.4%) *Culex* gravid females were collected in surveys 1 and 2 from the entire PHC area as against 16500 targeted for each survey and were distributed in 660 pools (165HH x 4 pools per HH). In the hotspot area, 7630 (99.6%) and 7595 (99.3%) *Culex* gravid females were collected as against 7650 targeted in each survey and were distributed in 306 pools (153HH x 2 pools per HH). The median pool size was 17 and 25 in the PHC area and hotspot areas respectively (Figure 1.5).

The mosquito pools are being processed to extract parasite DNA and to assess filarial infection

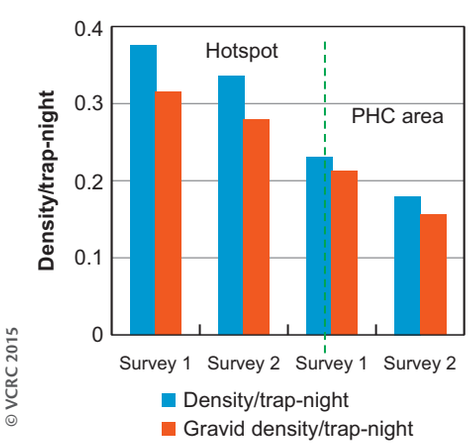


Figure 1.4 Comparison of *Culex* density in the hotspot and entire PHC area.

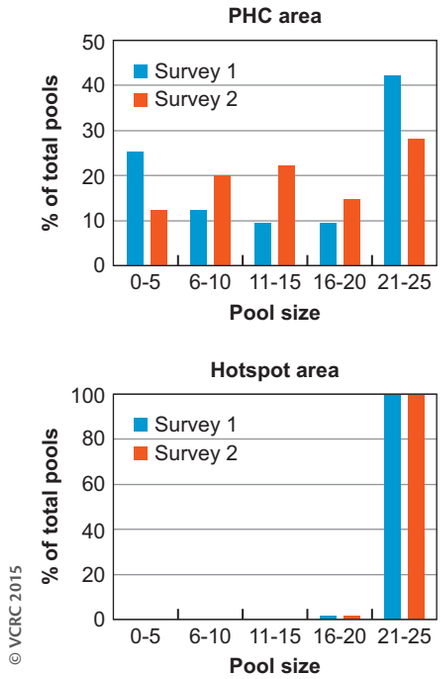


Figure 1.5 Distribution of number of gravid *Culex* females (pool size) by no. of pools and surveys in the PHC area and hotspots.

by RT-PCR. The data will be used to carry out simulated sample surveys for determining the optimum number of households and the number of pools per household.

A sample blood survey is proposed to assess the current status of infection (Mf and Ag) in the study area. The results of Mf and Ag prevalence in human will be compared with the prevalence of infection in vector to examine if vector infection could reflect the changes in human infection 8 years after stopping MDA. The funding agency has agreed to extend its support for this purpose.

1.1.7. Field validation of 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, Sivagnaname N, Jambulingam P, and Investigators from the Directorate of Public Health, Tamil Nadu, Puducherry, Goa

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 at sub-district level (Project ID: EM 1001). This study is to validate its usefulness/feasibility as an alternative to TAS for post MDA surveillance in operational settings in an evaluation unit.

Objective:

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

The study was carried out in Cuddalore district (implementation unit, IU), where the mass drug administration programme was in operation since 1996-97. A total of 12 rounds of MDA has been completed in this district. The IU consists of seven taluks (Cuddalore, Chidambaram, Kurinchipadi, Panrutti, Kattumannarkoil, Thittakudi, and Virudhachalam) having 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 Panrutti formed EU1 (population: 1.4 million) and the taluks Virudhachalam, Tittakudi and Kattumannarkoil formed EU2 (population: 1.0 million). Of the two EUs, EU1 was selected for the study. Details of population and number of households by village / ward (cluster) in each PHC / town in the EU1 were obtained from the Census Department.

For the purpose of mosquito survey, a two-stage village / ward (cluster) and household cluster sampling protocol was followed. In stage 1, 30 clusters were selected from 690 villages / wards in the EU. In stage 2, on an average 5 households (HH) per cluster were selected with probability proportional to size of the selected cluster (Figure 1.6). In each selected HH, gravid traps were fixed to collect mosquitoes. Mosquito collections in each HH were continued until a total of 50 *Culex* gravid females were caught or for a maximum of 3-4 nights. Mosquitoes collected from each HH were sorted to species and the *Culex* females were further classified into gravid and unfed. The gravid females from each HH were then distributed into two pools of 25 mosquitoes each. Thus altogether, it was planned to collect 300 pools totalling to 7500 *Culex* gravid females (i.e. 30 clusters x 5 HHs per cluster x 2 pools per HH x 25 mosquitoes per pool = 7500 mosquitoes).

A total of 14,642 female mosquitoes comprising 4 species were collected by spending 407 trap nights in 186 households spread over 30 villages/ wards in the EU (Figure 1.6). Of the mosquitoes collected, the filariasis vector, *Culex quinquefasciatus*, is the predominant species (13771; 94.1%), followed by *Armigeres* (3.8%), *Aedes* (1.4%) and *Anopheles* (0.8%). A similar pattern was observed in all the four taluks (>90% *Culex*). Of the 13771 *Culex* females collected 12525 (91%) are gravids. The mean *Culex* gravid density in the EU was 30.8 per trap-night (range: 24.5- 41.3 in the four taluks, Figure 1.7).

For the purpose of PCR assay, a total of 354 pools were made from the 8750 *Culex* gravid females as against the targeted 7500 gravids. The pool size was 25 in 99% of the pools (range: 5 to 25 in different pools). The mosquito pools are preserved and the PCR assay is in progress.

In order to have a parallel assessment of vector infection with Mf-prevalence, microfilaria survey is proposed in all the 30 sites in the EU, which will be completed within the project period. For this purpose, a total of 8500 persons need to be sampled from the 30 sites. Sample for each site will be allocated in proportion to the population size of the site. The sample size was calculated for an expected Mf-prevalence of 1% with 0.25% precision and 95% confidence interval.

In our earlier study, a vector infection rate of 0.6% was estimated to be equivalent to 2% Ag-prevalence in children of 6-7 years, the cut-off value for stopping MDA. The PCR assay results of this study will be compared with the above critical

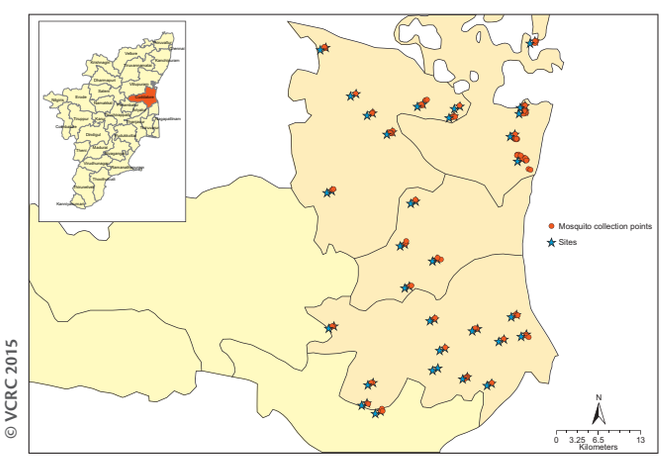


Figure 1.6 Location of sites (villages / wards) and mosquito collection points in the EU.

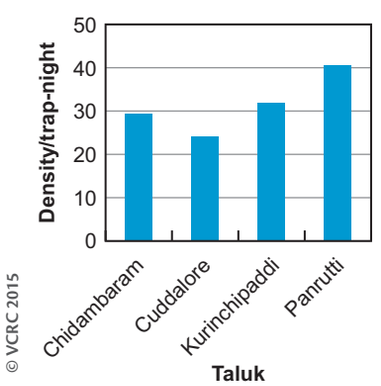


Figure 1.7 Taluk wise density of gravid *Culex quinquefasciatus* females collected in EU1 of Cuddalore district.

cut off value. For the same study area, the result of TAS is also available for comparison.

1.1.8. Adaptation, validation and application of LYMFASIM model to predict the risk of resurgence following stopping MDA based on transmission assessment survey (TAS)

EM 1509: Aug 2015 – Jul 2018

Subramanian S, Vasuki V, One Investigator from NVBDCP and Jambulingam P

Lymphatic filariasis (LF) is targeted for elimination by 2015 in India. Due to operational issues related to achieving the targeted compliance, there are indications that the programme requires to be extended. Persistence of infection even after 10 rounds of MDA in some of the IU's is a major challenge to the programme. The study aimed to help in (i) predicting the scope of accelerating the LF-elimination programme with strategy adjustments by considering increasing frequency of MDA per annum, other treatment regimens (3-drug regimens), supplementary measures (vector control, DEC salt), (ii) targeting 'hotspots' based on model predicted risk of residual transmission to elimination goal and (iii) choosing cost-effective surveillance methodology for monitoring the epidemiological situation after cessation of the ongoing elimination programme in the country for an early detection of risk of resurgence.

Objectives:

- To adapt and validate the epidemiological simulation model, LYMFASIM for India in response to changing diagnostic, treatment and surveillance data.
- To simulate progress made in LF elimination programmes, and estimate how long annual MDA would still need to be continued to achieve elimination.
- To assess the comparative effectiveness of adjusted strategies and added interventions to accelerate LF-elimination programmes and improve prospect for achieving LF elimination by 2020.

In this study, an individual-based micro-simulation model called 'LYMFASIM', is being used to address the above-mentioned objectives. LYMFASIM simulates a dynamic human population and transmission of infection between human individuals and mosquito population. The model accounts for several factors which are critical for predicting elimination through MDA, including individual heterogeneities in exposure to mosquito biting and compliance with MDA, stochastic effects contributing to elimination/recrudescence, and variability in diagnostic test outcomes in epidemiological surveys. The model keeps track of changes in infection status (e.g. number of immature and mature, male and female worms) at the individual level over time. Individual outputs are aggregated to provide output on population-level indicators of infection. The core biological parameters of the model were previously quantified by fitting the model to longitudinal entomological and epidemiological data from an integrated vector management programme carried out in Puducherry, India, from 1981–1986. The resulting model fitted well to the data, and also provided accurate estimates of trends in microfilaria (Mf) prevalence both before and after cessation of integrated vector management. During the reporting period LYMFASIM was used to (i) test hypotheses about the sources of filarial antigen as detected by the currently used immuno chromatographic card (ICT), (ii) estimate the required duration of MDA for different endemic settings, and (iii) estimate the 1-year post-MDA infection levels associated with successful elimination.

i. ***Testing hypotheses about sources of filarial antigen for ICT:*** LYMFASIM was extended with new functionality to predict an individual's Ag status based on his/her infection status as would be observed with ICT for detecting filarial antigen. This new feature in LYMFASIM was used to resolve prevailing uncertainities about the relative contribution of different parasite life stages to Ag levels and regarding the test sensitivity for the detection of amicrofilaraemic adult worm infections. Three hypotheses for the association between antigen and the presence of adult worms against empirical data were tested: Ag is detectable in the presence of at least (a) one male or one female worm, (b) one female worm or worm pair present; single-sex infections with male worms only remain undetected, (c) one male + female worm pair. The hypotheses were tested by simulating the impact of 8 annual rounds of MDA (4 with DEC and 4 with DEC+albendazole) on the prevalence of Mf and Ag. The model predictions were compared with the data collected from one of the PHCs in Thanjavur district, Tamil Nadu, which had the same MDA features.

ii. ***Estimating number of MDA rounds:*** LYMFASIM was used to estimate the required duration of MDA for achieving elimination (Figure 1.8). For this purpose, simulations were done to predict trends in different infection

indicators during and after MDA, for four epidemiological settings varying with respect to baseline endemicity: Puducherry setting, for which the model was originally quantified, and three hypothetical settings which only differed from Puducherry with respect to the monthly biting rates (mbr) of mosquitoes and hence the endemicity levels at baseline. The mbr in Puducherry was 2200, corresponding to a pre-control mean Mf prevalence of 8.5%. The hypothetical settings reflected communities with low transmission (mbr = 1600, mean baseline Mf prevalence 4.9%), medium transmission (mbr = 1950, mean baseline Mf prevalence 7.4%), and high transmission (mbr = 2700, mean baseline Mf prevalence 10.0%). The minimum number of MDA rounds required to achieve elimination was determined for each of the four epidemiological settings and for three levels of treatment coverage (50%, 65%, 80%). For each of the 12 epidemiological setting-coverage combinations, the expected trends in infection during and after MDA, for different durations of MDA (1, 2, 3, rounds) was simulated. In view of the stochastic variation between simulation runs, a total of 1000 repeated runs per scenario was carried out, all with the exact same input assumptions. For each run, the elimination status (success or failure) was recorded and was calculated the elimination probability per scenario as the percentage of runs that reached this outcome, with elimination defined as zero Mf prevalence 60 years after the start of MDA. The lowest number of MDA rounds that resulted in a ≥99% probability of elimination was taken as the required duration of MDA.

iii. ***Post-MDA infection levels associated with successful elimination:*** LYMFASIM used to assess the 1-year post-MDA levels for Mf and antigen prevalence that are associated with successful elimination in different endemic settings.

iv. ***Testing hypotheses about sources of filarial antigen for ICT:*** Figure 1.9 shows the goodness of fit of model to data of the Mf and Ag prevalence by age after 8 rounds of MDA (4 with DEC and 4 with DEC+ALB). The predicted Mf-prevalence is 0.22, 0.12 and 0.04 for 50%, 55% and 65% MDA-coverage respectively and the corresponding values for Ag-prevalence are 3.84%; 3.4% and 1.06%. The results indicate that the prediction based on a MDA coverage of 55% was found to approximate the observations made in this area (Mf-prevalence:95% CI=0.2%, 0.15–0.25%; and Ag-prevalence=2.2%,

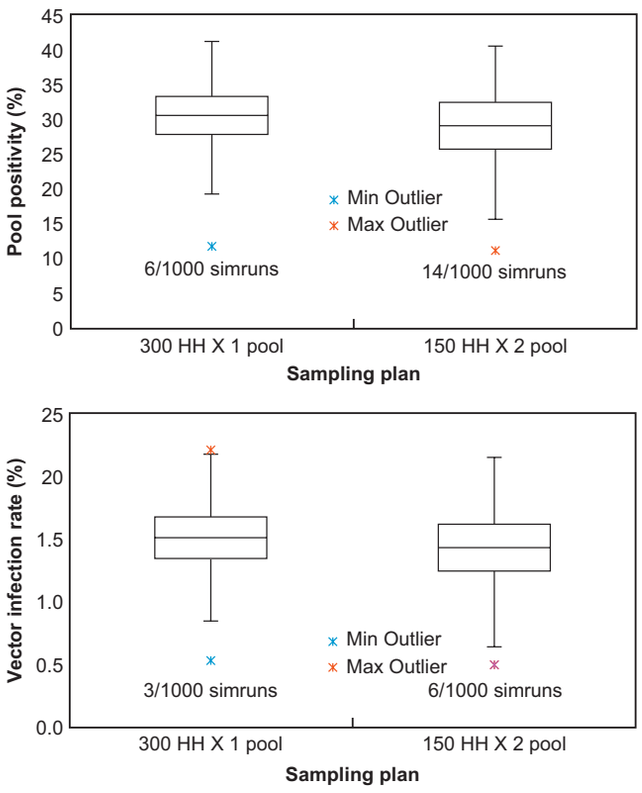


Figure 1.8 Comparison of simulated sampling plans by pool positivity and vector infection rates using data from all the 'PHC clusters'.

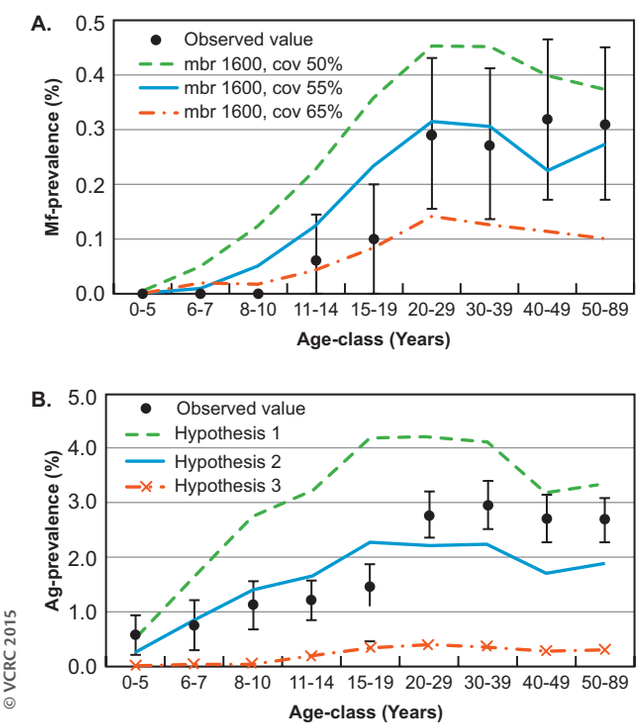


Figure 1.9 Goodness of fit of model to data of the Mf (A) and Ag prevalence (B) by age after 8 rounds of MDA (4 with DEC and 4 with DEC+ALB). MDA impact is simulated with varying levels of 'coverage' for a man biting rate of 1600.

2.05–2.35%). The age-patterns on Mf-prevalence could not exactly be reproduced, but the absolute level in adults adequately matched when a 55% MDA coverage per round was assumed (Figure 1.9(A)). Figure 1.9(B) shows that hypothesis 1 overestimates the Ag prevalence in all age-groups, while hypothesis 3 results in a strong underestimation. Predictions obtained under hypothesis 2 are in the right order of magnitude, although the levels in adults were somewhat underestimated. The overestimation of Ag prevalence in teenagers is balanced by the overestimated Mf prevalence in this age group. Based on these findings, the model was adopted with hypothesis 2 and was used for predicting levels of Mf and Ag prevalence prior to and one-year after last MDA.

v. ***Estimating number of MDA rounds:*** Table 1.8 shows the number of annual MDAs needed for achieving ≥99% probability of infection elimination for different endemic settings with varying treatment coverages. In low endemic situations the number of MDAs needed (2-4 rounds) is lower than in settings with intermediate (3-7) and high (4-12) baseline endemicity. The required duration doubled or tripled with decreasing coverage levels for all settings or increasing endemicity.

vi. ***Post-MDA infection levels associated with successful elimination:*** Figure 1.10 summarizes simulation results with respect to the Mf and Ag prevalence in the population aged 5 years and above, and 6-7 years prior to MDA and 1 year after the required rounds of MDA for elimination. The median residual Mf prevalence was 1.1% at the lowest mbr and 0.4% at the highest mbr; similarly, the median residual Ag prevalence was 6.8% at the lowest mbr and 2.8% at the highest mbr (Figure 1.10(A-D)). Qualitatively similar patterns were predicted for infection prevalence in 6-7 year old children (Figure 1.10(E-H)). The median residual Ag prevalence in this group was 3.5% at the lowest mbr and 2.0% at the highest.

Table 1.8 Number of annual mass treatments required to achieve ≥99% probability of elimination in relation to varying coverage and MDA

Endemic setting (Monthly Biting Rate)	No. of MDA rounds required at different levels of coverage		
	50	65	80
Low (1600)	4	2	2
Intermediate (1950)	7	4	3
Puducherry (2200)	8	5	3
High (2700)	12	6	4

The results suggest that to achieve elimination in high transmission settings, MDA must be continued several years longer and infection levels must be reduced to substantially lower levels than in low-endemic communities. This should be taken into account in decision algorithms to define when MDA can stop in a certain area. Further, where feasible, transmission assessment surveys should be targeted to communities with the highest pre-control transmission levels, to make sure that the duration of MDA has really been long enough to achieve the required residual infection levels.

Future work: LYMFASIM will be adapted with data on antibody assay from the same study area, from where the Ag-prevalence data was available (Thanjavur) as well as data from sentinel and spot-check surveys in different parts of the country. Further, LYMFASIM will be used to evaluate the comparative effectiveness adjusted strategies (MDA with triple drug regimen) and added interventions (DEC salt, vector control etc) to accelerate LF elimination programme and improve the prospect for achieving LF elimination by 2020.

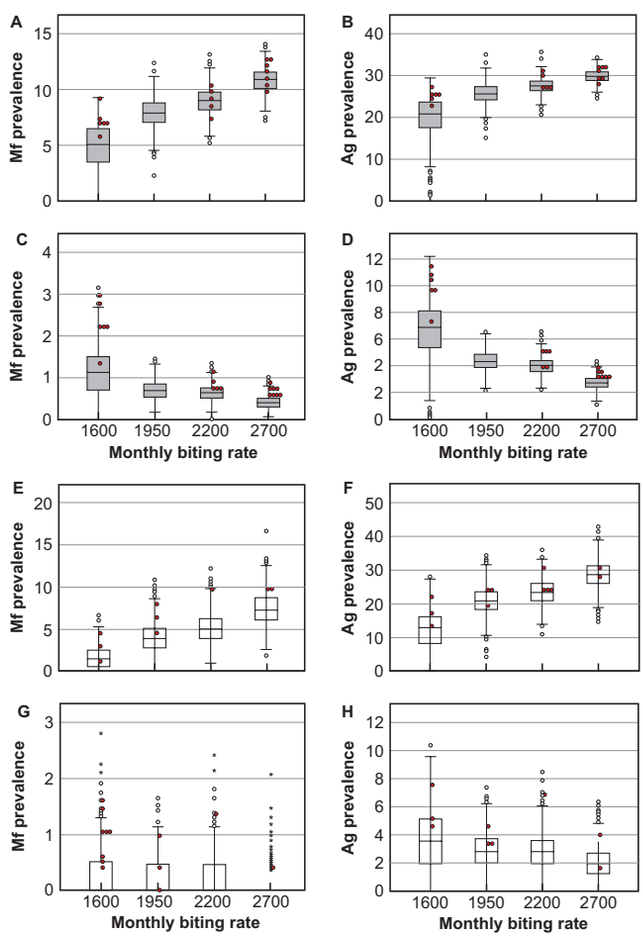


Figure 1.10 Predicated infection prevalence for the population aged 5 years (A-D) and above, and 6–7 years (E-H), prior to MDA and 1 year after the required treatment duration.

1.1.9. Development and demonstration of strategies to enhance community compliance for MDA for ELF in Palakkad district of Kerala state

EM 1511: Jan 2015 – Dec 2016

Nandha B, Vijayakumar KN, Meenakshy V, (NVBDCP, Kerala)

Eleven out of 14 districts are endemic for filariasis and are under MDA since 2004. Four districts have qualified for stopping MDA, another four districts qualified for Transmission Assessment Survey (TAS). MDA is continued in the remaining 3 districts. Among the 3 districts, Palakkad with 29.8 lakh population showed persistence of infection above 1% in six out of 8 sentinel/spot check sites. A 30% gap in compliance was reported in the 9th round. As the reasons for non-compliance are situation specific, micro-level observations are needed to identify the gap, determinant factors and develop appropriate strategies. Therefore, this study was undertaken. The 10th round of MDA was conducted in this district in December 2014 to January 2015 in two phases. In the first phase, MDA was implemented in 18 areas identified to have persistent transmission (17 PHCs and 1 Municipality) and in the second the remaining areas were covered.

Objectives:

- To identify the gap between coverage and consumption and the cause for the gap using Health Belief Model.
- To develop and demonstrate appropriate strategies based on the findings of objective 1.
- To evaluate the process and impact of MDA based on the intervention.

Study area: This is a community intervention study to improve the coverage and compliance with three arms. Palakkad district has urban, semi urban, rural and tribal areas with three arms. As many as 4 wards from 2 municipalities, 5 Semi urban PHCs, 10 rural PHCs and 2 Tribal PHCs were selected randomly for the study. From each of these PHCs, one sub centre area with four wards was also randomly selected. The sample size for the qualitative studies was calculated based on the expected drug consumption rate of 20% and confidence level 95%. Thus, 676 individuals in 21 areas were subjected to detailed qualitative interviews and information on consumption was gathered for 3806 individuals. All the systematic non compliers available in the selected 676 households (527) and 210 drug distributors were also interviewed. As many as 42 Key informants (2 per site) and 42 focus group discussions (one with women and one with men in each site) were also conducted.

Study design: Based on the drug consumption rate in the 10th round of MDA, the 21 sites were stratified into three arms so that each stratum consists of six villages and one ward. A joint action plan has been prepared with the state programme to strengthen both drug distribution and social mobilization activities in arm I and II. VCRC was involved in capacity building/training/social mobilization to optimize MDA activities according to the recommended National Guideline in Arm II. Additionally, site specific activities such as involvement of small groups in the community, strengthen existing groups like Kudumbasree and Ayalkoottam for MDA and creation of a network of the groups for community mobilization towards compliance to MDA will be carried out under research mode by VCRC in Arm I. Routine activities as per the national guidelines will be carried out in Arm III for comparison without any additional inputs from VCRC. Participant observation of MDA, assessment of MDA as per guideline and detailed qualitative and quantitative studies carried out pre-intervention will be repeated post intervention.

To assess the impact of planned interventions, microfilaria survey will be carried out jointly with the Health Dept of Kerala, in 9 randomly selected sites (6 villages and 3 wards), 3 each from each of the three arms is also planned. The sample size is calculated based on expected Mf prevalence of 1%, confidence limit 0.5 and confidence level 80%. The impact will be assessed 6 months post MDA in the same areas.

Results: Participant observation showed that MDA 2014 was conducted through DOT in group gatherings in public places and through door to door distribution using ASHAs, Anganwadi workers, Health staff, volunteers and nursing students. Monitoring of 36 such group gatherings in 10 wards and 18 villages revealed that a standard protocol of drug distribution was lacking. Observation of activities of 61 drug distributors indicated that the drug distributor's training was focused on the technical aspect of MDA but lacks the steps to be followed in motivating the community. Lacunae in the supply of materials including shortage of Albendazole, poor quality (powdered) DEC tablets, expiry date crossed Albendazole tablets and late arrival of IEC materials in the concerned PHCs showed that planning of MDA activities needs improvement.

Coverage of drug distribution and consumption was assessed in 2 urban areas and 6 villages following the national guideline. Quantitative and qualitative surveys were carried out in 21 sites (4 wards of

2 municipalities and one village from 17 PHCs). The results of these surveys indicated that out of 3806 individuals interviewed, 67.6 % (range 58-81) had received the drugs, 32.5% (range 18-53) consumed the drugs. Compliance was 48.4%, ranging from 40-66 in urban areas. The same for rural areas was 70.5% (range 48-94), 35.4 (range 4-62) and 50.2 (11-83). Both coverage and consumption were below the target level of 85% and 65% respectively.

The results of the in-depth interviews showed that, though 67% had awareness on MDA programme, the need for every individual to comply with the programme was not felt by the community which shows that there exists a gap in preparation of the community. Systematic non compliers constituted 20% and the reasons attributed by majority (72.8%) of them were seen to be modifiable determinants. Interviews with drug distributors showed that though all had essential knowledge on MDA, the barriers in smooth functioning is poor remuneration and distance to be covered. The analyses of the detailed studies are in progress.

The interventional activities of formation of small groups in the community, strengthening of the existing groups and networking between groups for the smooth functioning of MDA is ongoing in all the 7 areas in Arm I and will be continued till the subsequent MDA planned in December 2015. Intensive Behaviour Change Communication classes have been conducted in all the 7 areas and 28 such meetings have been conducted jointly with the health department of Kerala.

Mf surveys have been completed in all the 9 areas. The Mf rate for the 3 areas carried out initially was found to be 1.4% and in the remaining sites slide observation is in progress.

1.1.10. Using Intervention Mapping to accelerate Mass Drug Administration compliance to achieve Lymphatic Filariasis elimination in areas with persistent transmission in India

EM 1512: Dec 2015 – Apr 2017

Nandha B, Krishnamoorthy K, Sharma SN, (NVBDCP, Bihar)

Though appreciable progress has been made to achieve the national goal of elimination of lymphatic filariasis (LF) as a public health problem by 2015, a few endemic districts are still highly endemic with persistent transmission. Bihar, Andhra Pradesh and Gujarat have 38, 16 and 11 endemic districts respectively with more than 1% Mf prevalence. We propose to apply the Intervention Mapping stepwise approach for theory and evidence based development of interventions for implementation with reference to improving compliance rate.

Overall objective:

- Development of theory and evidence-based intervention to accelerate compliance with MDA and submitting to the programme for implementation.

Specific objectives:

- To understand the gap in the coverage and identify the cause for the gap between coverage and compliance using behaviour determinant theories.
- To develop appropriate strategies in each cultural context.

One district each with Mf prevalence above 1% will be selected from three states, Gujarat, Telengana and Bihar.

Study area: Muzaffarpur district is endemic for filariasis and Mf survey carried out in 2005 showed 8.5% Mf rate. As many as 10, 119 lymphoedema and 7,790 hydrocele cases have been enumerated. Mf survey carried out in 2013 in 4 sentinel and 4 spot-check sites, with 500 samples from each of the sites showed that five sites showed more than 1% Mf rate. The population of this district is 49, 52,055 (updated in 2013) and there are 2, 51,671 households. This district was brought under elimination programme in 2004 and so far seven rounds have been completed. There are 16 CHCs with 505 sub-centres and 1889 villages. A total of 5033 drug distributors were used with a target of 1000 individuals for each drug distributor.

MDA was carried out from 28th May 2015 for 15 days in Muzaffarpur district and was assessed based on the reported coverage. One village each from three PHCs namely Bochaha, Motipur and Kanti with High, Medium and low coverage and one ward from the urban area was selected. From each area, 30 houses were randomly selected with a minimum of 150 individuals/area and information was collected for 747 individuals in 120 houses using the standard format.

Out of 747 individuals interviewed in 120 households from one urban ward and 3 villages, 43.8 % (range 29.3–65.2) had received the drugs, 16.3% (range 7.6–33.7) consumed the drugs and compliance was 37.3% (range 23.6–51.7). In the urban ward, coverage, consumption and compliance was 41.7%,15.6% and 37.3%. Both coverage and consumption were below the target level of 85% and 65% respectively indicating the need for situation specific targeted approaches.

Detailed studies will be carried out to identify the gap and create matrices for intervention objectives. A brainstorming session with expert group will be conducted to identify behaviour change techniques, that are feasible, locally relevant, and acceptable. The identified components will be combined into an acceptable intervention for implementation by programme people in the concerned districts.

1.1.11. 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

RMRC Post Blair: Shriram AN, Scientist B
VCRC: Krishnamoorthy K, Directorate of Health Services, Andaman & Nicobar Administration, Port Blair: Amitabh De, Deputy Director and Avijit Roy, Deputy Director (Health)

General objectives:

- To demonstrate mass delivery of DEC fortified salt as a supplementary measure to the ongoing annual rounds of MDA (DEC+albendazole) towards elimination of the lone foci of diurnally sub-periodic *Wuchereria bancrofti.*

Specific objectives:

- To evolve a plan for DEC fortified salt supply and putting in place a delivery mechanism.
- To assess the operational feasibility through appropriate indicators and community compliance.
- To assess the impact of this programme in terms of epidemiological indicators and compare with MDA alone.
- To carry out costing of the DEC fortified salt programme supplemented in the elimination of diurnally sub periodic filariasis.
- To conduct post intervention Transmission Assessment Surveys including xenomonitoring for the risk of resurgence and final evaluation for the certification of elimination.

The baseline survey for assessment of microfilaria prevalence, antigenemia prevalence among children and salt usage pattern of the community in the five Nancowry group of islands were completed (Annual Report 2014) and the islands were grouped and randomized into two arms, one for intervention (Teressa and Nancowry islands, population: 3055) and the other for comparison (Chowra and Kamorta islands; population: 4929).

As many as 2170 *Do. nivea* female mosquitoes have been collected from Nancowry, Kamorta, Teressa and Chowra islands. Since BG sentinel traps are not suitable in the forest areas, double net collection method was followed. A total of 217 pools, each with 10 mosquitoes have been made available for processing by PCR. Double fortified salt with iodine and DEC was procured from Tamil Nadu Salt Corporation, a fully owned enterprise of Govt. of Tamil Nadu. The Corporate supplied 24 metric tons of double fortified free flow salt in one Kg packets. Shipment of salt has already reached Nancowry islands.

A joint effort in developing appropriate social mobilization strategy has been made to maximize coverage and compliance in using double fortified salt. Proforma for monitoring the process has been finalised and to be pre-tested.

The programme was formally launched on 20.11.2015 by Chief Secretary, Andaman & Nicobar Administration.

Community volunteers will be selected to distribute salt in each of the selected islands for distribution of DEC salt. Methodology to monitor salt usage and community compliance has been developed which will be followed for process evaluation.

Double fortified salt manufactured by TNSC

1.2 MALARIA / LEISHMANIASIS

1.2.1. Entomological and epidemiological investigations on cutaneous leishmaniasis among the Kani tribes in Thiruvananthapuram Dt. Kerala

EM 1206: Jun 2012 – Jul 2015

Srinivasan R, Sabesan S, Pradeep Kumar N, Paily KP and Jambulingam P, Dilip Kumar, Nandakumar S*, Anish TS***

**State Health Department, Thiruvananthapuram*
***Govt. Medical College, Thiruvananthapuram*

The Kani tribes, one of the oldest ethnic groups of India are scattered over the hilly tract of the southernmost part of the Western Ghats in Thiruvananthapuram dt., Kerala. Recently cutaneous leishmaniasis (CL) cases have been reported from the tribes. However, the extent of leishmaniasis among the tribes was not known due to the lack of accessibility, communication and transport. To plan and implement interventions, it was essential to assess the extent of prevalence and incidence of leishmaniasis cases among the Kani tribes, vector(s) involved in the transmission and the factors associated with the emergence of leishmaniasis cases. Hence, entomological and epidemiological investigations on leishmaniasis with cutaneous manifestations were carried out in the Kani tribe settlements.

Objectives:

- To assess the prevalence and incidence of cutaneous leishmaniasis among the *Kani* tribes.
- To incriminate vector species of sandfly(s) involved in transmission of cutaneous leishmaniasis.
- To study the seasonal abundance, resting and feeding behaviour of sandfly vector(s).
- To assess *Leishmania* infection among animal reservoir(s).
- To assess the inter-relationship among the climatic, demographic and biotic factors with sandfly prevalence.

During the year, the project was completed and final report submitted. The summary of the project is given below. In a cross sectional survey conducted in 28 tribal settlements, sandfly abundance showed a negative correlation ($r = -0.97$, $p = 0.003$) with increase in altitudinal ranges between 267 and 2,425 ft. Sandfly species showed great aggregation < 900 ft altitude interval, where not only the number of settlements were maximum (n = 19), but also the environmental conditions favoured sandfly abundance due to the concentration of tribal settlements, human dwellings and their activities.

Out of the total 1,444 Kani tribe population, clinical examination was done on 341 males and 427 females for cutaneous leishmaniasis (CL) infection and 27 CL/suspected cases were recorded. Healed lesions with scars due to CL infection were noticed in two settlements namely, Melaamala (N = 11) and Ayiramkal (N = 1). The remaining (n = 15) cases were recorded from Keezheamala (n = 10), Kaithode (n = 2), Podium (n = 1), Melaamala (n = 1) and Kombidi (n=1), who had either nodule(s) or active lesion(s). Five tissue/ dermal pulp samples collected from human cases were found positive for *Leishmania* infection based on nested PCR amplification of kinetoplast mini-circle DNA. On analysis of the sequences, all the samples were found to be connected 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 obtained were KC884001, KF562068–KF562071.

A total of 15,940 sandfly specimens, comprising of 4,852 males and 11,088 females was obtained during longitudinal survey (June 2012 – May 2014). Percentage contribution of *Phlebotomus argentipes* population from human dwellings, cattle sheds and outdoor habitats was 52.8%, 37.6%, and 9.6% respectively, (n = 3,288). The corresponding figures for sandfly species other than *P. argentipes* were 83.6%, 0.0% and 16.42%, respectively (n = 12,652). A total of 19 sandfly species was recorded. *P. argentipes* (18.64%) was the second predominant species indoors, next to *S. baghdadis* (36.52%). While all the species were recorded indoors, only 16 were from outdoors. Sandfly density recorded from indoors was significantly higher than that of the outdoors.

P. argentipes population showed a major abundance peak in October, which received a maximum rainfall during northeast monsoon. However, climatic conditions influenced the population. Multiple regression analysis indicated that the average monthly precipitation and relative humidity contributed significantly to the prediction of abundance of *P. argentipes* population ($F = 23.37$; $R2 = 0.84$, df = 2.9, $p = 0.001$), while there was no significant correlation with minimum and maximum temperature ($p > 0.2$).

Density of resting populations of *P. argentipes* (no. of females/MHR) at various height intervals in human dwellings differed significantly during summer (one-way ANOVA: F=83.7, df=5.12; p=0.001) and rainy season (F=41.4, df=5.24, p=0.001) but not in cooler months (F=1.67, df=5.18, p=0.2). During summer, the density was higher (p<.05) at the height levels of 6-8 ft (55.7 females/MHR) and >8 ft including ceiling (48.3 females/MHR) than in other height levels. In rainy season, the density was higher at 0 ft (33.6 females/MHR) and 0-2 ft (20.0 females/MHR). However, in cooler months, the density did not show any variation, in relation to height of the wall.

A total of 919 sandfly females comprising *P. argentipes*, *P. colabanensis* and *P. stantoni* were subjected to PCR assays, to detect natural infection. Among the three species tested, natural infection with *Leishmania* parasites were found in 7 pools, comprising of only *P. argentipes* females. On sequencing, the parasites were identified to be *Leishmania donovani*. *P. argentipes* has been incriminated to be the vector of cutaneous leishmaniasis from the Kani tribes. The hSP-70 sequences amplified from sand fly females were sequenced and deposited in the GenBank (Accession numbers: KR905363 - KR905367).

Sandfly females (n=120) were tested for insecticide susceptibility. The corrected mortality for field collected *P. argentipes* population with DDT as well as deltamethrin at diagnostic concentrations was 100% after 24 hr of exposure. Other species, though in small numbers were also found susceptible to 4% DDT and 0.05% deltamethrin.

A total of 47 blood samples collected from domestic dogs were subjected to both PCR assay and ELISA tests. Three samples were positive for *Leishmania donovani* infection in PCR assay, while five in ELISA test. This has been confirmed through sequencing. A total of 25 rats, comprising two species viz., *Rattus rattus* and *R. norvegicus* were collected. Samples collected from these rats were tested through PCR assay and none was found positive to CL infection. Blood smears and tissue smear (liver and spleen) were examined microscopically and all were found negative for infection.

At the request of the Directorate of Health Services, Govt. of Kerala, a training programme on entomological and parasitological aspects of cutaneous leishmaniasis was conducted to the personnel of the District Malaria Offices of Thiruvananthapuram, Kozhikode, Palakkad and Thrissur districts. A practical demonstration on indoor residual spraying activity in sandfly-infested areas was conducted. Based on insecticide susceptibility status, seasonal abundance and resting behaviour of sandflies, application of indoor residual spraying (IRS) with either DDT or synthetic pyrethroid in entire interior surface of the human dwellings, twice a year (first round during April-May and second round during September-October) was recommended to the DHS, Govt. of Kerala to implement in the tribal area.

1.2.2. 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 2016

Jambulingam P, Gunasekaran K, Sahu SS, Subramanian S, Behera KP & Swati Kumari**
**State Health Department*

Though IVM in NVBDCP 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 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 study aims to devise strategies for optimal use of these two measures in combination (Arm 1, LLIN+IRS) or singly (Arm 2, only LLIN & Arm 3, only IRS) through integrated approach and to make a comparative assessment of the intervention arms on mortality and morbidity due to *fluviatilis* transmitted *falciparum* malaria.

Objectives:

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

This is a quasi-experimental study carried out in an area where the state NVBDCP has already been implementing the intervention measures. In this study, the Arm 1 includes 86 villages under eight sub-centres (SCs) with a population of 32,966 in Laxmipur CHC and 88 villages under 8 SCs with a population of 33,969 of the same CHC are included in Arm 2. Arm 3 has 99 villages under 7 SCs with a population of 25,616 in Podagada

sector of Dasmanthpur CHC. In Arm 1 and Arm 2, LLINs were distributed during July–August 2012 and in Arm 1 and 3, yearly two rounds of indoor residual spraying with DDT has been carried out; thus Arm 1 is with two intervention measures.

The study is conducted in two phases. During Phase 1 (Nov 2013–Oct 2014), data on entomological (density, survival and human blood index) and parasitological (parasite incidence) parameters were collected from the index villages of the three arms (Arm 1: 8 villages, Arm 2: 8 villages & Arm 3: 7 villages) - Annual Report 2014. During Phase 2 (November 2014–October 2015), with the additional inputs by VCRC on IEC activities for the improvement of coverage and compliance, implementation and evaluation are continued in the three arms.

Spray coverage: The room spray coverage during Phase 1 and 2 in the Arm 1 and Arm 3 is shown Figure 1.11. During Phase 1, the spray coverage in Arm 1 was 46.8% (range: 38.9–66.9%) and 50.7% (range: 37.5–77.6%) during round 1 and 2, respectively; the corresponding values for Arm 3 were 47.2% (28.1–64.7%) and 42.0% (32.7-51.3%). During Phase 2, Round 1, the spray coverage was 35.1% (range: 28.0%–38.9%) in Arm 1 and 42.5% (ranged from 32.9%–50.9%) in Arm 3; in Round 2, the corresponding values were 55.0% (range: 35.5%–64.9%) and 55.5% (range: 52.3%–59.3%). The spray coverage was improved during the II Round of Phase 2 in both the arms.

Net usage and status: The survey conducted in Arm 1 and 2 during May 2015 i.e. after three years of distribution of LLINs showed that out of 24,274 LLINs distributed (against the target of 26774 LLINs), 12,050 (49.6%) nets were in usable condition. The rest of the LLINs were either damaged or missed. The net use rates in Arm 1 ranged from 54.4 to 61.4% during Phase 1 and 41.8 to 54.8% during Phase 2. The corresponding values for Arm 2 were 51.5–55.0% and 46.5%–50.0%.

Surveillance and treatment by ASHAs: A total of 25 ASHAs from each of the three arms were interviewed for testing their knowledge on malaria diagnosis and treatment, knowledge on components of diagnostic kits, prevalence of malaria parasite species, drug regimen for different parasite species and age-classes and blood smear collection using a questionnaire and LQS method. Since, only <8 ASHAs answered correctly (Cut off: 8), a training programme focusing on malaria diagnosis and treatment and drug regimen was organized to all ASHAs in the study villages. The survey conducted after the training indicated an improvement of their knowledge and performance by 30%.

Cone bio-assay for insecticide effect of LLINs: Since adequate number of *An. fluviatilis*, the susceptible vector, could not be collected from the study area, *An. jeyporiensis*, another susceptible species, was used for bio-assays. After 30 months of distribution, 16 LLINs obtained from 16 randomly selected villages (one net from each village) were subjected to cone-bioassay; the mosquito mortality was 97.5% (n=750). Cone bio-assay after 36 months of distribution (20 LLINs from 16 randomly selected villages; one net from each village) showed only 77.5% (n=400) mortality.

Vector abundance indoors & outdoors: The density (number per man-hour) of *An. culicifacies* and *An. fluviatilis* recorded in human dwellings, cattle sheds and outdoors in the three arms are shown in Figures 1.12–1.17. Two way analysis of variance (ANOVA) showed that the changes in density of the two vectors occurred between Phase 1 and 2 did not vary significantly among the three arms

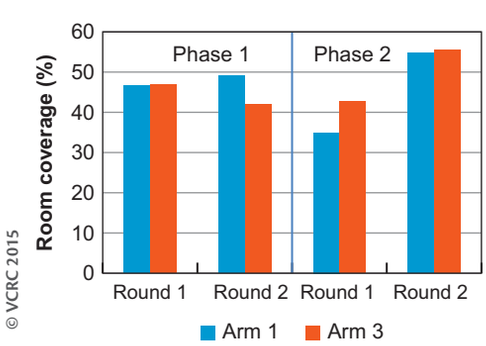


Figure 1.11 Room spray coverage in Arm1 and 3.

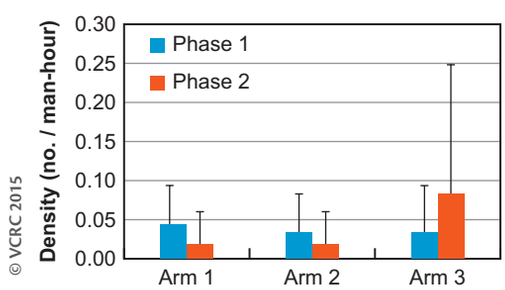


Figure 1.12 Density of *An. culicifacies* in human dwellings.

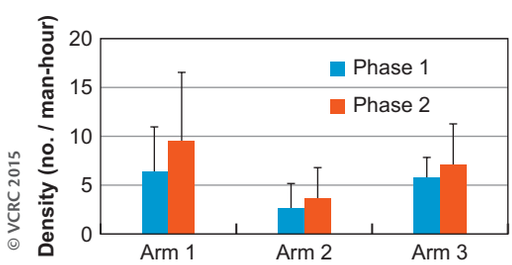


Figure 1.13 Density of *An. culicifacies* in cattle sheds.

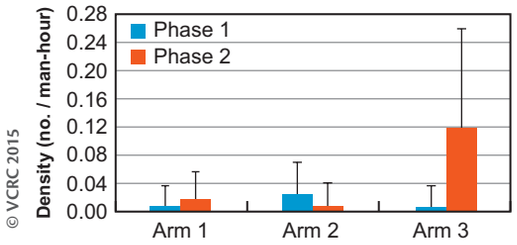


Figure 1.14 Density of *An. culicifacies* in outdoors.

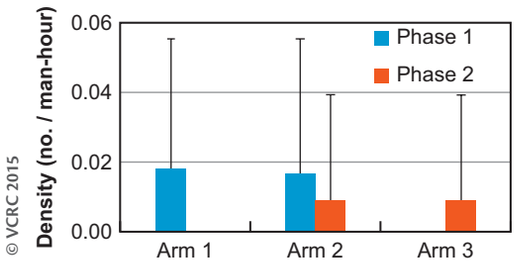


Figure 1.15 Density of *An. fluviatilis* in human dwellings.

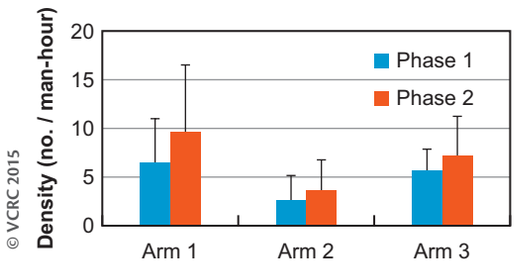


Figure 1.16 Density of *An. fluviatilis* in cattle sheds.

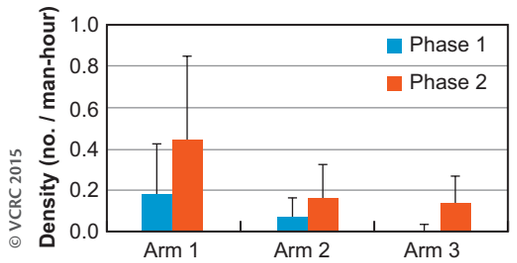


Figure 1.17 Density of *An. fluviatilis* in outdoors.

(interaction effect: P > 0.05), except the density of *An. culicifacies* outdoors in Arm 3 was significantly higher than the other two arms (interaction effect: P = 0.004; 95% CI of arm 3 did not overlap with the other two Arms).

Parous rate: The changes in the parous rate over the Phases 1 and 2 (Table 1.9) was compared between the three arms using logistic regression analysis. The increase in parous rate of *An. culicifacies*in Arm 1 was significantly different from Arm 3 (P = 0.04) but not from Arm 2 (P = 0.43). In the case of *An. fluviatilis*, the changes in parous rate over the two phases was not significantly different between the arms (P > 0.05).

Human blood index (HBI): *An. culicifacies* was predominantly zoophagic with 97.8% to 100% during Phase 1 and 98.6 to 100% during Phase 2 of its blood meals were positive for bovine blood. Almost similar results (Phase 1: 100% and Phase 2: 99.0% to 100% in the three arms) were obtained for *An. fluviatilis* in all the three Arms. The change in HBI of the two species over the two Phases was not significant between the arms.

Malaria parasite species & incidence: *P. falciparum* was the predominant species (about 94%) and around 6% was due to *P. vivax* infection. The annual parasite incidence (API)recorded during Phase 1 and 2 in the three arms are shown in Figure 1.18. The API in individual arms did not differ significantly between phase 1 and 2 (chi-square test, P > 0.3 for all comparisons). Also, the changes in the API observed over Phase 1 and 2 was not significantly different between the arms (logistic regression; Arm x Phase: P=0.76)

The vector parameters and incidence of malaria did not differ significantly between the three arms over the study period, Phase 1 and Phase 2. The coverage and compliance of the two intervention measures are not adequate in the three arms; the number of usable nets and

Table 1.9 Parous rate of the vector species during Phase 1 and 2

Species	Arms	Phase 1		Phase 2	
		Number dissected	Parous rate (%)	Number dissected	Parous rate (%)
An. culicifacies	Arm 1	588	42.7	768	50.0
	Arm 2	260	44.2	315	47.6
	Arm 3	489	45.4	541	44.0
An. fluviatilis	Arm 1	117	23.1	178	39.9
	Arm 2	35	25.7	71	39.4
	Arm 3	9	11.1	50	28.0

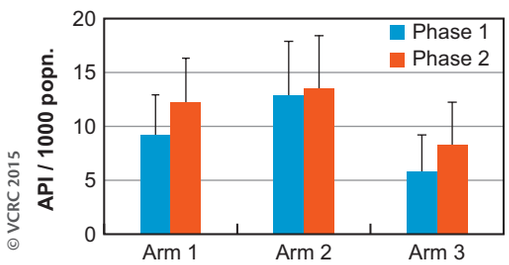


Figure 1.18 Annual parasite incidence (API) in the three Arms.

use rate have come down during Phase 2 compared to Phase 1. The insecticidal effect of the LLINs reduced from 97% to 77% in Arm 1 and Arm 2 during the Phase 2. With the given level of coverage and compliance, the combination of measures may not add any additional benefit over single measure. In order to achieve the desired impact on vector parameters and malaria incidence,the coverage and compliance need to be enhanced through further strengthening of IEC activities.

1.2.3. Morphological and molecular taxonomy of the *Phlebotomus argentipes* species complex in relation to transmission of Kala-azar in India

EM 1506: Aug 2015 – Jul 2017

Srinivasan R, Jambulingam P (VCRC), Gowri Shankar J, Arun Kumar KP (CDFD, Hyderabad), Pradeep Das, Dinesh DS (RMRI, Patna), Ilango K (ZSI, Chennai)

One of the National Health Policies of India is "Kala-azar elimination" by 2020 (revised). Efforts are being made to achieve the goal of elimination through IVM. The number of kala-azar cases is showing a reduction in Bihar and West Bengal. However, in a few states such as Jharkhand and Uttar Pradesh the cases are slightly increasing. Sporadic cases are recorded in Gujarat, Assam and Kerala. *P. argentipes* population involved in kala-azar transmission shows the existence of cryptic species. To support the existing intervention measures and to target the member species among the complex, a study has been initiated.

Objectives:

- Morphological and molecular characterization of *P. argentipes* species complex in endemic and non-endemic areas.
- Detection of natural infection of *Leishmania donovani* among the members in *P. argentipes* complex.

Based on sandfly abundance/kala-azar cases/ cutaneous leishmaniasis cases, one village from each of the following districts viz., Vaisali, Jaguakanj, Mungar and Gaya in Bihar, 24 Parganas in West Bengal, Gorakhpur in Uttar Pradesh, Ranchi in Jharkhand, Pune and Mahabalishwar in Maharashtra, Puducherry in Puducherry UT and Thiruvananthapuram and Thrissur in Kerala, is selected for the study.

Sandfly surveys were carried out in villages of Bihar, Puducherry and Pune. In each of the samples of *P. argentipes* population collected, a portion (head, wing and spermathecae of female specimens) is subjected to morpho-taxonomy and the remaining part (thorax and abdomen) is used to identify *Leishmania* infection if any, blood meal source (if the females were found with fresh blood meal) and sibling species, using molecular markers. The preliminary result indicates occurrence of variations in morphometric parameters among *P. argentipes* population collected from Bihar and Puducherry. Further study is in progress.

1.3 DENGUE / JE / KFD

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

EM 1208: Nov 2012 – Oct 2016

Jambulingam P, Rajavel AR, Subramanian S, Gunasekaran K

Baseline data collection on entomological parameters, commenced in August 2013 was continued through 2014 and 2015 in Campierganj and Belghat (intervention blocks) in Gorakhpur district and in Majhgawa (comparison block) in Deoria district.

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.

Fortnightly indoor and outdoor resting collections were made in the study villages Bahadurpur, Bharsi, Barigaon, Harpur and Gayghat of Belghat block, villages Sarpatha, Machligaon, Ramnagar, Shivpur and Kaharpurwa of Campierganj block and villages Katrari, Pidara, Lahilpar khas, Babhani and Saraura in Majhgawa block. 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 (HBI). Both the samples were transported to the laboratory at VCRC for processing and analysis.

Vector density (PMD): 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 1.19. During the period of three years from 2013 to 2015, the vector density exhibited a similar trend in all the three blocks with higher densities observed during the months of July, August, September and October. The peak density occurred during September-October in 2013 and during August-September in 2014 and 2015. The indoor density of *Cx. tritaeniorhynchus* was generally lesser than the outdoor density. The density of the other two vector species, namely, *Cx. vishnui* and *Cx. pseudovishnui* was almost negligible in all the three blocks.

Blood Meal Index (BMI): A total of 2097 samples were collected and analyzed for blood meal identification. The BMI for human, bovine and pig were respectively 0.004, 0.902 and 0.043 in 2013; 0.005, 0.946 and 0 in 2014 and 0, 1, 0 in 2015 in Campierganj Block. For the Belghat Block it was 0.008, 0.935, 0.014 in 2013; 0, 0.827, 0.066 in 2014 and 0, 0, 0 in 2015. In the comparison Block Majhgawa, it was 0.002, 0.972, 0.002 in 2013; 0, 0.707, 0.028 in 2014 and 0, 1, 0 in 2015. Blood meal analysis has shown that the JE vector *Cx. tritaeniorhynchus* predominantly feeds on cattle.

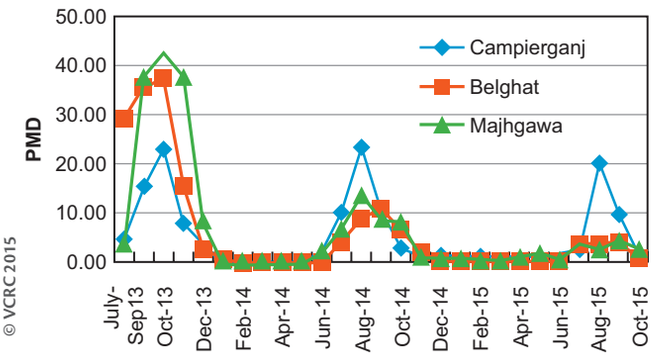


Figure 1.19 Per man-hour density of *Culex tritaeniorhynchus* in Blocks Campeirganj, Belghat and Majhgawa (July-August 2013 to October 2015).

Minimum Infection Rate (MIR): For JE virus detection in mosquitoes, a total of 6621 mosquitoes (in 417 pools) from Campierganj and Belghat intervention blocks of Gorakhpur district and Majhgawa control block in Deoria were collected and analyzed. A total of 9 pools in the intervention blocks and 11 pools in the control block were positive for JEV. MIR for intervention and comparison blocks respectively was 4.33 and 4.07 in 2013, and 0 and 2.22 in 2014.

The intervention protocol was to carry out indoor residual spray (IRS) with Lambdacyhalothrin 10% WP at 25mg/sq.m. in 2 Blocks, namely, Belghat and Bhathat and to distribute Long Lasting Insectidal Net (LLIN) in 1 Block, namely, Campierganj. Majhgawa block of the Deoria district will remain as control for comparison.

Spray coverage: The indoor residual spray in Belghat was commenced on 1st June 2015 and completed on 29th July covering the 28 sub-centers while in Bhathat it was commenced on 2nd June 2015 and completed on 13th July covering the 27 sub-centers. A total of 39,537 houses in 205 villages and 34,581 houses in 240 villages were sprayed in Belghat and Bhathat respectively. House spray coverage of 92.2% and room spray coverage of 88.68% was achieved in Belghat while

in Bhathat it was 93.03% and 87.36% respectively. Besides human dwellings, a total of 16,196 cattle sheds and 42 pig sties in Belghat and 8.270 cattle sheds and 72 pig sties in Bhathat were also covered by the indoor residual spray.

LLIN distribution: Distribution of LLIN was commenced on 12th August 2015 in Campierganj, but it was possible to cover only 4 sub-centers since it had to be suspended due to the logistic problems as a consequence of the gram panchayat elections. It is proposed to resume the LLIN distribution in the month of December.

Post-spray evaluation: The per man hour density of the vector species, *Culex tritaeniorhynchus* in Belghat Block, following the indoor residual spray was lower than the density recorded during the same period in the previous year. However, the density in the comparison block Majhgawa remained comparable till the month of September 2015, but in the month of October 2015 the density recorded in Belghat was lower than that of Majhgawa. Analysis of a total of 177 blood meal samples and a total of 620 mosquitoes in 91 pools collected during the post spray period is in progress for determining the BMI and MIR.

1.3.2. Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan regions of Himachal Pradesh and Jammu & Kashmir

EM 1505: Aug 2015 – Jul 2018

Rajavel AR, Pradeep Kumar N, Natarajan R, Shazia Wafai & Jambulingam P

Information on the diversity and distribution of mosquitoes in the Himalayan region dates back to the 1970s. The mosquito fauna of the states of Himachal Pradesh and Jammu & Kashmir have not been updated since then, but the region has gone through much environmental changes, which would have resulted in changes in the floral and faunal diversity. Changes in species diversity will have a bearing on the threat of vector borne diseases and hence it is necessary to document the current species diversity in this region. The project aims to fulfill this need by documenting the mosquito fauna and their distribution in relation to altitude in the Himalayan region of Himachal Pradesh and Jammu & Kashmir.

Objectives:

- To document the mosquito fauna of Himachal Pradesh and Jammu & Kashmir.
- To update the information on mosquito species diversity of the region.
- To determine the mosquito species distribution in relation to altitude.
- To DNA barcode the different species recorded in the region.

Mosquito faunistic survey was done during the months of August and September 2015 in Jammu & Kashmir to document the species diversity. The State is comprised of three divisions namely, Ladakh, Ka shmir and Jammu. Collections were done in Phyang, Basgo, Hemis, Thiksay, Shey, Horje, Horje Gompa, Ganglas, South Pullu and North Pullu in Leh district where altitude ranged from 10490-16000 ft., and in Sankoo, Lankerchey, Lankercheythang, Khachan, Baroo, Barchey, Lalung, Batalik, Umbolung, Budhkharbu, Namikala, Shargol, Kurbathang and Drass in Kargil district where altitudes ranging from 9300–12460 ft. in Ladakh division. In Kashmir division, collections were done in Uri, Boniyar, Silikot, Uroosa, Kamalkote, Suldandaki, Dachi, Saidpora and Lagama in Baramulla district; Saidakadal, Nigeen lake, Habha Crossing, Laam Nishat, Lashker and Naseem Bagh in Srinagar district; Vaibagh, Ganiwan, Wangath and Narang in Ganderbal district; Kaitch, Raiyar and Doodhpathri in Budgam district; and Dogripora in Pulwama district where altitude ranged from 3868 – 8366 ft. In Jammu division, collections were made in Mishriwala, Akhnoor and Mansar in Jammu district and in Patnitop, Phalata, Battal, Trilla, Kirmoo and Kaghote in Udhampur district where altitude ranged from 895–6495 ft. adult collections were done in human dwellings, cattle sheds and outdoor (bushes in forest, river margins, stream margins, pit shelters and tree bases) habitats by using oral aspirator and sweep net. Larval collections were carried out in a variety of habitats that included swamps, ground pools, irrigation canals and seepages, streambed pools, stream margins, riverbed pools, river margins, rock pools, tree holes, bamboos, cement tank, ponds, roadside pools, plant axils and discarded tyres.

A total of 1238 adults obtained from resting collections and those emerged from larval collections were pinned and a total of 567 larvae, larval and pupal skins were mounted on slide from which 34 species in 10 genera were recognized. Thirteen species were collected in Ladakh division which included 7 species of *Culex*, 1 species of *Aedes*, 3 species of *Ochlerotatus* and 2 species of *Culiseta*. Of the 13 species, 7 species namely, *Ae. stenoetrus*, *Cx. modestus*, *Cx. pusillus*, *Cx. quinquefasciatus*, *Oc. caspius*, *Oc. pulchriventer* and *Cs. indica* were first time records

from Ladakh. In Kashmir division, 22 species were collected, of which 5 species, namely, *Cx. bitaeniorhynchus*, *Cs. niveitaeniata*, *Oc. oreophilus*, *Lutzia halifaxi* and *Coquillettidia perturbans* were first time records. The record of *Cq. perturbans* is significant as it is a new country record for India. In Jammu division, 11 species were collected of which *Cx. minutissimus* and *Malaya genurostris* were first time records. Voucher specimens of all the species collected have been deposited in the mosquito museum at VCRC.

1.3.3. Studies on the transmission dynamics and control of Dengue in forest fringe areas of Kerala

IM 1303: Sep 2013 – Aug 2016

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

The studies were initiated during 2013 and baseline information on the risk factors associated with the transmission of dengue in Kanjirappally taluk was studied during the first two years. This taluk bordering Western Ghats is considered as the epicenter of dengue in Kerala state and the first case of dengue in the state was reported from this taluk. Since then, the incidence of dengue had been on an increasing trend in the region, every year. The study area include two villages in the forest fringe area and two wards in Kanjirapalli urban area, having a population of approximately 1800–2970.

Objectives:

- To study relative abundance of *Stegomyia* 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 dengue outbreaks in these ecosystems.

Vector population: Twenty two species of mosquitoes were recorded in Kanjirappalli region (n=12421). The most predominant species was found to be *St. albopicta* (39.46%). While, both species of Stegomyia vectors, *Stegomyia aegypti* and *St. albopicta* were found prevalent in the urban area, only *St. albopicta* was recorded in the rural forest fringes. The peak of abundance of these species was recorded during pre-monsoon summer in the both settings (Figure 1.20 & 1.21). For *St. aegypti* this could be attributed to the acute water storage in the urban area, which lead to storage of water in large containers. These habitats contributed 83.38% of the population of this species in the urban area. Breeding sources of *St. albopicta* was found to be diverse and these proliferated due to intermittent rainfall during the summer season (Annual Report. 2014).

Dengue infection in vector population: Natural infection with DENV infection was recorded only in *St. aegypti* during the study period. A total of 463 specimens of *St. aegypti* (266 females & 197 males) collected as adults / immatures were processed during the study period in 234 pools. The specimens were segregated according to abdominal conditions (UF, FF, SG & G). Out of 234 pools of *St. aegypti*, two pools of SG (June & July 2014) were found positive for DENV infection. The positive samples were from indoor resting collections. One of the pools had DENV4 and another pool was with DENV2 infection. The results were communicated to the Dept. of Health services and as a rapid

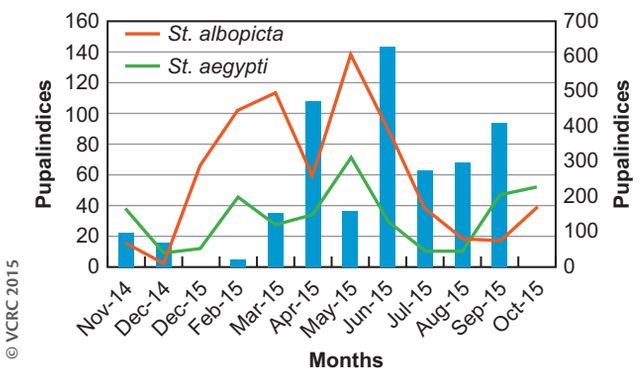


Figure 1.20 Vector population density (pupal indices) in the urban area (Kanjirappally town).

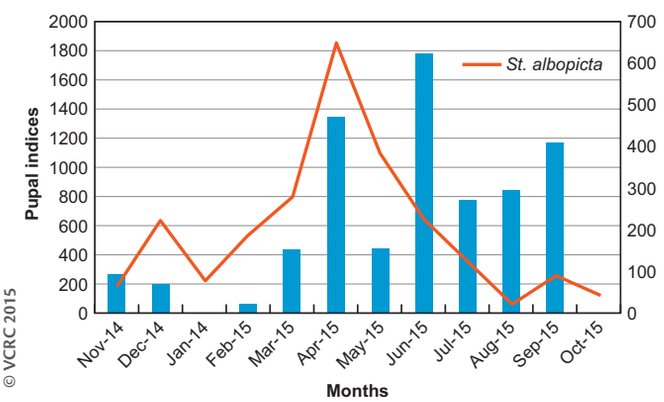


Figure 1.21 Vector population density (pupal indices) in the rural forest fringe villages of Kanjirappally.

response, fogging was carried out to prevent transmission. None of the 653 (147 pools) specimens of *St. albopicta* processed from the urban area were found positive for DENV.

St. albopicta females collected from rural areas were also screened for arbo-viral infection. None of the 142 pools (n=433) processed showed positive for DENV infection.

Blood meal analysis: DNA barcode analysis was done to determine the source of blood meal of the mosquitoes. 46 specimens (*St. aegypti* – 20; *St. albopicta* – 26) were subjected to COI sequence analysis and all of these were found to be from *Homo sapiens*.

Incidence of Dengue: Hospital based surveys during 2013-2014 revealed a reduction in the incidence of disease (per 100,000 population) from 231.60 to 112.28 during these years. The reported cases recorded by IDSP were found to be comparatively less due to non-reporting of cases who attended private medical institutions. No case of infection was recorded from the study villages during the study period.

The results of the investigations carried out indicate that the foci of transmission in the taluk could be the Kanjirappally urban area, where the *St. aegypti* population was found. Vector infection in *St. aegypti* was recorded during the peak season during both the years of survey. The key vector breeding habitats of this species was water storage containers (85.79%) which were maintained in each household in the urban areas with water scarcity during summer season.

Management of water scarcity problem in collaboration with LSG authorities could be the permanent solution for vector management in the area. Community education campaign to prevent storage of water in open containers for more than a week period could be carried out involving community Health workers, students and self-help groups as Anganwadi and Kudumbashree volunteers etc., during the summer season to prevent dengue outbreaks in the area.

1.3.4. 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 & Nandha B

In view of increasing trend of *Aedes* borne arboviral diseases, this study was undertaken to develop and demonstrate integrated vector management (IVM) focusing on fostering inter-sectoral collaboration and empowering communities for the prevention and control of dengue in two PHCs viz., Mannadipet and Ramanathapuram in Puducherry with population of 15331 (9 villages). One PHC (Thirukanur) with a population of 10528 in five villages is the control arm. Dengue cases are reported every year in these PHCs. Community Readiness assessment study in these villages indicated low level community readiness in undertaking preventive measures, demanding a system for constant motivation.

Objectives:

- To establish collaboration and networking among various inter-sectoral partners through partnership.
- To identify risks and methods of preventing vector breeding.
- To prepare community – create awareness and mobilize their participation.
- Develop a system for continued motivation, monitoring community action and entomological surveillance.

High levels of environmental risk (79.9% of the households with vector breeding sources) with a mean number of 3 habitats per house were observed during baseline (2013). 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 Neighbourhood committees were identified and involved as potential stakeholders in motivating the community and mobilizing their participation besides monitoring the risks of vector breeding. Five schools in four villages were involved under school based approach. From each school 25 students were involved for fortnightly visits. Clearing discarded containers/utensils, discouraging the practice of discarding utensils/ containers (rain dependent) and managing the water storage containers (cisterns and plastic drums) are the key messages given by the school students. The results indicated that only school based

approach was operationally feasible and sustainable and therefore this approach alone was continued during 2015. Self-help groups, NGOs, and Neighbourhood committees were very irregular in collection of data and could not be relied upon as a reporting system. The villages allocated for other approaches were monitored as control villages. Monthly entomological surveillance was carried out in all the four villages under school based approach and 15 villages without any intervention.

Students visited the respective villages at fortnightly intervals till April 2015. Following the closure of schools for vacation, this exercise was discontinued to assess the sustainability of community action. A total of 620 households were visited by students during 2015 and observed that 26% and 56 % of the houses had discarded and water storage habitats as source of vector breeding. In the comparison area as many as 1679 household were visited by research team and observed that discarded containers were found in as many as 51.1% of the households while water storage sources were found in 64.5% of the households examined. The percentage of houses with vector breeding was only 1.6% in the intervention area compared to 3.6% in comparison area. The proportion of houses with discarded containers was significantly lower in the villages where school based approach was followed. The mean number of discarded habitats was 0.7 per house in school based approach while it was 0.8 in the comparison area. The discarded containers in the comparison area was significantly higher in comparison area (3.5 per household) compared to 2.3 in the villages under school based approach. Though all the entomological indicators showed lower values in the villages under school based approach, the indicators started increasing during the period when the visits of students were discontinued, indicating that continuous motivation is required to sustain community action.

In view of developing a sustainable surveillance system, a collaborative approach has been designed with the school education department. School students were trained on assessing the breeding sources in and around their houses. A simple pictorial assisted proforma in local language was prepared. A total of 472 students were trained. About 75% of the students could complete the forms and the monitors in the class were given preliminary training on the distribution, collection and verification of forms for its completeness. Fortnightly surveys are proposed. A teacher from each school has taken responsibility to ensure that the activity is continued. As a follow-up activity, a training workshop at the Centre is planned to train two monitors in each of the selected class and a teacher from these study areas will be conducted jointly with State NVBDCP.

Every school is equipped with a computer with internet facilities. Online transmission of consolidated data on entomological parameters will be sent every fortnight so as to enable the State NVBDCP to monitor the situation and wherever and whenever interventions are indicated, appropriate action will be carried out by the state NVBDCP. Ministry of Local Administration and Department of School education have already committed to this joint collaborative venture. The study is in progress.

1.3.5. Preliminary studies on 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

Sadanandane C, Raju KHK & Elango A,
Co-ordinators: Jambulingam P & Mourya DT (NIV)

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 above potential risk areas.

During the reporting year, collection of ticks species was carried out in the following areas: (i) Pulpally and Sulthan Bathery forest ranges of Wayanad district, Kerala where outbreaks of human KFD cases were reported in 2014-2015, (2) Nilambur and Nedumkayam reserve forests (Nagamalai hills) in Malappurram district of Kerala where KFD virus in both humans and monkeys was detected in 2014, (3) four sites at Mudumalai Tiger reserve in Nilgiri district, Tamil Nadu where KFD virus was detected from autopsy of dead monkeys in 2013 and (4) two sites at Sathyamangalam Reserve forest in Erode district of Tamil Nadu

where evidence of KFD virus circulation was not reported until 2015. In each of the selected site, ticks from the forest floor were collected using lint clothes (100x70 cm) by flag dragging method during January and May 2015.

The species diversity of ticks collected in Malappurram and Wayanad districts of Kerala and Nilgiri and Erode districts of Tamil Nadu is summarized in Table 1.10. A total of 4403 tick specimens belonging to 8 species of genus *Haemaphysalis*, two species each of genus *Ambylloma*, *Boophilus*, *Rhipicephalus* and one species of genus *Ixodes* was collected. In all the districts surveyed *Haemaphysalis spinigera*, the primary vector of KFD virus was the predominant tick species collected. *Haemaphysalis turturis*, the other major vector of KFD virus was also recorded in all the districts surveyed which constituted 7.7% of the total tick specimens collected. Other *Haemaphysalis* species collected were *H. bispinosa*, *H. intermedia*, *H. cupsidata*, *H. wellingtoni*, *H. aculeata* and *H. pupua kinnaeri*. *Ambylomma* species formed 5.6% of the total ticks collected (Table 1.10). All the tick samples (pooled in 213 vials) were labeled and coded and the coded specimen samples were kept in liquid nitrogen containers and sent to the NIV, Pune for laboratory testing for evidence of KFD virus infection. Virus isolation by tissue culture is being carried out at NIV, Pune.

Table 1.10 Species diversity of ticks collected in Malappurram and Wayanad districts of Kerala and Nilgiri and Erode districts of Tamil Nadu

S.No.	Species	Wayanad	Malappuram	Nilgiri	Erode	Total
1	*Haemaphysalis spinigera*	508 (47.7%)	468 (39.3%)	821 (60.4%)	519 (65.9%)	2316 (52.6%)
2	*H. turturis*	94 (8.8%)	94 (7.9%)	70 (5.1%)	81 (10.3%)	339 (7.7%)
3	*H. bispinosa*	209	198	8	0	415
4	*H. intermedia*	2	0	0	23	25
5	*H. cupsidata*	3	3	0	0	6
6	*H. wellingtoni*	4	0	0	0	4
7	*H. aculeate*	0	2	0	0	2
8	*H. papua kinnaeri*	0	1	0	0	1
9	*Ambylloma integrum*	0	4	0	0	4
10	*Ambylomma sp.*	50	85	79	29	243
11	*B. annulatus*	0	4	0	0	4
12	*B. microplus*	42	0	0	0	42
13	*Rhipicephalus haemophysaloides*	1	0	0	0	1
14	*Rhipicephalus sp.*	6	5	14	0	25
15	*Ixodes sp.*	0	15	0	0	15
16	*Haemaphysalis sp. larvae*	145	313	368	135	961
Total		**1064**	**1192**	**1360**	**787**	**4403**

1.4 MICROBIAL / CHEMICAL AGENTS FOR VECTOR / PARASITE CONTROL

1.4.1. Development of monoterpenes extracted from the seeds of *Trachyspermum ammi* as macrofilaricidal composition (Translational Research)

EM 1125: Jul 2011 – Jun 2015

Nisha Mathew, Paily KP & Kalyanasundaram M

This project was undertaken to assess *in vitro* and *in vivo* macrofilaricidal activity of monoterpenes and different combinations of monoterpenes present in the fruit extract of *Trachyspermum ammi*.

Objective:

- To assess the *in vitro* and *in vivo* macrofilaricidal activity of *Trachyspermum ammi* fruit extract containing monoterpenes against filarial worms.

The seeds of *T. ammi* were extracted by two methods *viz.*, (i) by soxhlet extraction with methanol followed by solvent removal by rotary vacuum evaporation under reduced pressure (ME) and (ii) by hydro distillation using Clevenger apparatus (HD). The presence of four monoterpenes TE-1-4 in both extracts were identified by TLC and HPLC analysis, and the two extracts and four monoterpenes were individually screened for adulticidal activity against *S. digitata in vitro* (Annual Report 2011). Eight combinations (MCT 1-8) containing monoterpenes were prepared and screened against adult *S. digitata in vitro.* Among the two extracts, four terpenes and eight combinations, two combinations *viz.*, MCT-6 & MCT-7 exhibited higher macrofilaricidal activity with ED_{50} value of 0.006mg/ml (Annual Report 2012).

Preliminary *in vivo* screening of MCT-7 was carried out against adult *B. malayi* transplanted in experimental animal. When the drug was given orally we could not get the same results as observed in the *in vitro* tests. This may be due to the low bioavailability of the active components in the drug due to poor aqueous solubility or fast metabolism (Annual Report 2013).

During the reporting period (2014-2015), MCT-6 and MCT-7 were formulated as self-emulsifying drug delivery systems (SEDDS) which could improve the oral bioavailability of poorly soluble drugs by improving the solubility and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract. The preliminary experimental results showed that the formulation was effective in killing the adult female filarial worms in infected *M. unguiculatus* when the drug (MCT-6 SEDDS) was given orally at 100mg/Kg body wt. However, at higher concentrations of 300mg/Kg body wt and 500 mg/Kg body wt the drug was not showing activity. Further *in vivo* animal experiments will be carried out in CDRI, Lucknow, in view of the limited facilities at VCRC.

1.4.2. *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: Jan 2012 – Dec 2015

Paily KP & Kalyanasundaram M (Retired)

In our attempts on development of macrofilaricidal compounds, substituted phenyl acetyl/benzoyl piperazides were synthesized and found to have activity against adults of cattle filarial worm, *Setaria digitata*, under in vitro conditions. In the present project, six of the most effective compounds were further screened under *in vivo* conditions against adults of human 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.
- 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, only three of the citrate salts of 1-N-methyl-4-substituted benzoyl piperazides (3-Methyl and 4-Methyl, coded as B7, B8 and B14) were effective against *B. malayi* in mongolian gerbils (*Meriones unguiculatus*). However, on further testing against *B. malayi* in multimammate rat (*Mastromys coucha*), through oral administration of 200 mg/kg, B7 and B8 also were found to be ineffective in killing adult worms (Annual Report, 2014). Hence, citrate salt of B14 (3,5-Dimethyl), which showed adulticidal activity in mongolian gerbil, alone was synthesized further and tested *in vivo* using multimammate rats as animal model.

Multimammate rats were infected with *B. malayi* by inoculation of infective larvae harvested from *Aedes aegypti* (Liverpool strain) mosquitoes fed artificially on mf positive blood samples. The mosquitoes after infection was maintained at room temperature for growth of the parasite to L3 and the harvested L3s were inoculated subcutaneously to animals so that the parasite larvae could migrate to different organs of the animal and develop to adults. After a pre-patent period of 120 days, blood smears of the animals were examined for the presence of mf and those positive for mf were used for testing of the compound (Table 1.11).

Mf positive multimammate rats were administered orally with citrate salt of B14 at 200 mg/kg body weight consecutively for five days. Two animals were given 400 mg/kg at divided doses in the morning and evening. Animals administered with normal saline were maintained as negative controls and those with DEC (200 mg/kg) were maintained as positive control. The animals were routinely monitored for weight loss, food intake and body temperature. They were sacrificed 90 days after administration of the drug and examined for the presence of adult worms located in various organs and microfilariae in the blood. Adult worms could be recovered from animals treated with B14 at 200 mg/kg body weight, as observed in the normal control animals, indicating that this compound is not effective in killing adult filarial worms when given orally. However, at 400 mg/kg, 100% mortality of the worms could be obtained.

Though B14 could exhibit complete mortality of adults at 400 mg/kg, the dosage is very high to be advocated as a drug for human consumption. The reason for the low activity of the compound could be due to its low level of absorption and its availability to lethal level to the worms located in different organs.

Table 1.11 In *vivo* antifilarial activity of citrate salt of 3,5-Dimethyl (B14) multimammate rats infected with *B. malayi*

Compound (dosage/kg)	No. of L3s inoculated	No. of worms recovered		Mf count/ 20 µl of blood (day 0–day 90)
		Male	Female	
B14 (200 mg)	68	1	1	40–291
B14 (200 mg)	80	1	0	405–242
B14 (200 mg)	42	0	1	159–280
B14 (200 mg)	70	1	1	37–184
B14 (400 mg)	50	0	0	766–899
B14 (400 mg)	89	0	0	1110–609
DEC (200 mg)	70	0	1	9–1
DEC (200 mg)	125	3	1	171–30
DEC (200 mg)	90	2	3	23–8
Control (I)	82	2	3	122–177
Control (II)	39	1	0	29–73
Control (III)	112	2	1	4–24
Control (IV)	125	2	1	53–291

1.4.3. Evaluation of bottle bio-assay for monitoring insecticide resistance in adult vector mosquitoes

IM 1503: Jan – Dec 2015

Gunasekaran K, Vijayakumar T, Sahu SS & Raghavendra K

WHO tube test has been the conventional method of determining susceptibility/resistance status of vector species. Though, it is the standard test giving satisfactory results, the kits have to be imported from abroad and are sometimes cost-prohibitive. Therefore, in order to have an alternative method for monitoring vector resistance to insecticides, the NIMR, based on the CDC bottle assay method, has developed a bio-assay technique using locally available bottles, which is simple to use in the field. This assay method was evaluated at VCRC against laboratory and field strains of mosquito populations in comparison to the WHO tube method.

Objectives:

- To evaluate bottle bio-assay method in determining susceptibility/resistance of mosquito species.
- To compare bottle bio-assay results with that of the WHO tube method.

Laboratory strains of *Aedes aegypti* and *Anopheles stephensi* and field populations of *An. jeyporiensis* and *An. culicifacies*, collected from the villages of Koraput district (Odisha State), were tested for their susceptibility to deltamethrin by exposing them in the bottles coated at the diagnostic concentration of 2 and 10 µg/ bottle, respectively. Six replicates for each replicate assay, four for test and two for control, were tested against each concentration, releasing 10-15 mosquitoes into each bottle. Knock down/mortality was recorded every 5 min for 1 hr. The mosquitoes were then kept in holding tubes and corrected mortality was calculated after 24 hrs of exposure. The tests were repeated three times on different occasions for each species. Parallel tests were done against impregnated paper of deltamethrin 0.05% (diagnostic dosage) using WHO tube method.

In the bottle assay, the criterion recommended for designating a population as susceptible is 100% mortality and a population as resistant is <100% mortality at 1 hr post-exposure (In the WHO method, the corrected mortality after 24 hrs of one hour exposure has been the criterion).

Table 1.12 Susceptibility / resistance status of mosquito species as determined in bottle bio-assay and WHO tube test

Species	Number exposed		Number KD_{60}		% KD_{60}		Number dead after 24 hrs of exposure		Corrected mortality (%)
	T	C	T	C	T	C	T	C	
Ae. aegypti (Lab. strain)									
Bottle bio-assay	180	90	180	0	100	0	180	0	100.0
WHO tube method	300	150	300	0	100	0	300	0	100.0
An. stephensi (Lab. strain)									
Bottle bio-assay	182	90	182	0	100	0	182	0	100.0
WHO tube method	300	150	300	0	100	0	300	0	100.0
An. jeyporiensis (Field strain)									
Bottle bio-assay	161	73	161	0	100	0	161	0	100.0
WHO tube method	156	78	156	0	100	0	156	0	100.0
An. culicifacies (Field strain)									
Bottle bio-assay	178	90	178	0	100	0	178	0	100.0
WHO tube method	156	78	76	0	48.7	0	126	1	80.8

In total (adding the three tests), 180 and 300 *Ae. aegypti* females of the laboratory strain were exposed to deltamethrin using bottles and WHO tubes, respectively. In both the methods, the % knocked down at 60 minutes (% KD_{60}) and the % corrected mortality (after 24 hrs holding) were 100%. Similar results were obtained for the laboratory strain of *An. stephensi* (no. exposed=182 & 300) and the field strain of *An. jeyporiensis* (no. exposed=161 & 156), indicating that the two methods were matching for the three species, which could be designated as susceptible to deltamethrin. However, for the field strain of *An. culicifacies*, the results of the two methods were not matching. In bottle bio-assay, while both % KD_{60} and % corrected mortality were 100%, in the WHO tube test the corresponding values were 48.7% and 80.8% (Table 1.12). With these results, it would be difficult to decide whether *An. culicifacies* is susceptible or resistant to deltamethrin. Therefore, diagnostic concentration for the bottle assay against *Anopheles* species needs further standardization.

1.4.4. DNA finger printing of *Bacillus thuringiensis* subsp. *israelensis* (VCRC B-17) strain, development of an improved production process/formulation and a real time PCR assay for quantification of delta endotoxin

IM 1502: Apr 2015 – Mar 2018

Manonmani AM, Prabakaran G, Geetha I, Sankari T, Muthukumaravel S, Mathivanan A & Jambulingam P

Bacillus thuringiensis subsp. *israelensis* (Bti) has been used as a mosquito larvicidal agent. VCRC has earlier developed a soya based production media for an indigenous strain (VCRC B17) and a process for formulating the cell mass into Aqueous Suspension (AS) formulation. This technology has been transferred to 11 commercial firms till date and soon is expected to be available for mosquito vector control programmes. Hence, it becomes imperative to monitor the environmental persistence of the bio-pesticide, differentiate it from other *Bti* strains and also quantify the concentration of crystal toxin available in the sites of application. Secondly, it is desirable to further reduce the production and operational cost by looking for alternative cost effective media and designing formulations with longer residual activity.

Objectives:

- To identify alternative, cost effective raw materials for production.
- To develop formulations with longer residual activity.
- To develop DNA fingerprint of VCRC-B17 based on different molecular markers.
- To develop a real time PCR technique for the detection of crystal toxin.
- To develop an ELISA for quantification of crystal toxin.

Thirteen different nutrient sources containing carbon and nitrogen sources were taken for media optimization of *Bacillus thuringiensis* var *israelensis* (VCRC B-17) (Table 1.13). Among these, 6 were selected based on its activity against *Culex quinquefasciatus* larvae (Figure 1.22) and cost of the media. The LC_{50} values (nl/100 ml) for K7, KD8 and KL9, KF10, T1 and W4 were 333, 351, 398, 402, 595, 623 respectively (Figure 1.23), while that of conventional medium (NYSM) was 669. The best among the 6 media combinations will be selected on the basis of production conditions in the fermentor.

A slow release floating formulation of *Bti* was prepared by mixing the active ingredient (*Bti*) with

Table 1.13 Nutrient sources tried for the production of mosquitocidal toxins from *Bti*

S.No.	Code	Common name	Botanical name	Cost/lit
1	T1	Pigeon pea	*Cajanus cajan*	2.20
2	D2	Double beans	*Phaseolus vulgaris*	4.00
3	U3	Black gram	*Phaseolus mungo*	2.20
4	W4	White pea	*Pisum sativum*	1.20
5	M5	Maize (corn)	*Zea mays*	3.00
6	MS6	Maize (Jowar)	*Sorgham bicolor*	1.60
7	K7	Bengal gram whole	*Cicer arietinum*	2.00
8	KD8	Green gram	*Vigna radiata*	2.60
9	KL9	Horse gram	*Macrotyloma uniflorum*	1.40
10	KF10	Bengal gram (broken)	*Cicer arietinum*	1.48
11	WF11	Wheat flour	*Triticum aestivum*	1.00
12	N12	NYSM	NYSM	30.00
13	S13	Soya flour	*Glycine max*	2.40

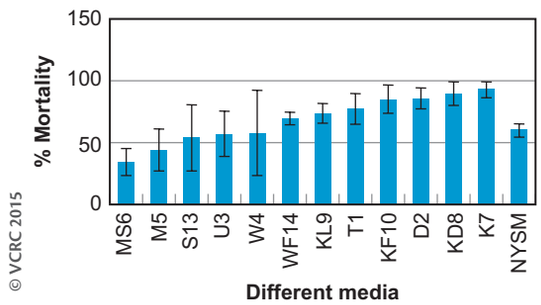


Figure 1.22 Percentage mortality incited among the larval stages of *Culex quinquefasciatus* by toxins of *Bti* (VCRC B-17) produced in different media.

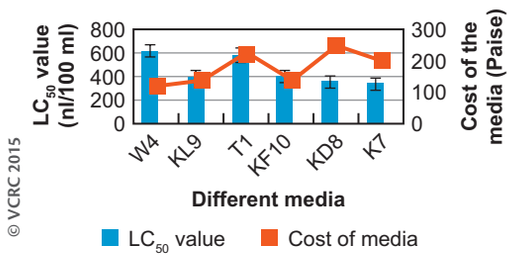


Figure 1.23 Efficacy of mosquitocidal toxins produced in the selected media including its cost.

pre gelatinized starch solution. This was later added with cork powder and formulated into balls of diameter 10 to 12 mm using a metal mould designed for this purpose. The formulation was tested in cess pits breeding *Cx. quinquefasciatus.*

Three and 4 numbers of cess-pits breeding *Cx. quinquefasciatus* were selected at Cuddalore and Villupuram districts respectively. They were treated at a dosage of 0.5 g/m^2, 1.0 g/m^2 and 1.5 g/m^2 of the formulation. Among the 3 dosages, 0.5 g/m^2 did not show any reduction up to 2 weeks, while the dosages 1g/m^{2}and 1.5 g/m^2 gave above 80% among the late instars by 10th and 7th day respectively which

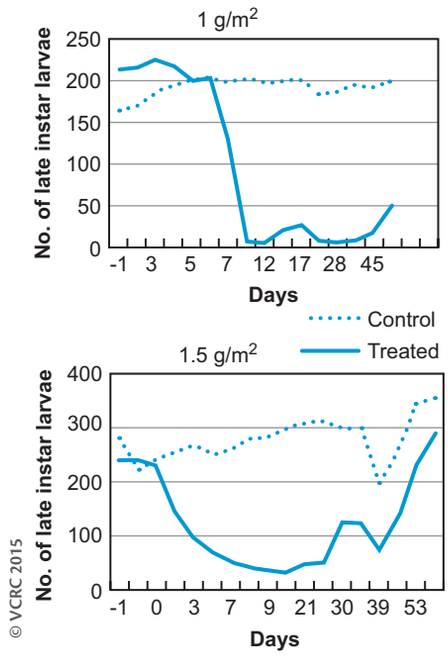


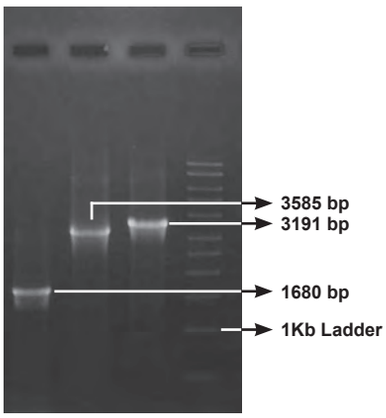
Figure 1.24 Efficacy of *Bti* slow release formulation in Cess-pits treated at 1.0 and 1.5 g/m2 against late instar stages of *Culex quinquefasciatus*.

was maintained for up to 45 and 25 days respectively (Figure 1.24).

DNA fingerprinting of Bti (VCRC-B17) was initiated using taxonomical marker genes and endotoxin genes. Taxonomical marker genes such as 16S, 23S rDNA, gyrB and RNA polymerase beta subunit (rpoB) were selected as they have been reported to be used for identification of bacterial strains and species. Since the insecticidal activity depends on the major endo-toxins of the toxin complex, endo-toxin genes Cry4Aa and Cry4Ba specific for dipterans were also selected by fingerprinting. Primers were designed based on the chromosomal (5.4 Mb) and plasmid (224 Kb) nucleotide sequence of Bti HD789 strain retrieved from GENBANK database using primer 3.V0.4.0 tool. Genomic DNA was extracted from VCRC B-17 and PCR was optimized and amplification of complete genes was achieved for 16s rDNA, 23s rDNA and rpoB with fragment size of 1680 bp, 3191 bp, 3585 bp respectively (Plate 1). Similarly, Plasmid DNA was extracted from Bti VCRC B-17 and the PCR for crystal endo-toxin genes of Cry4Aa and Cry4Ba was optimized and amplified with fragment size of 4358 bp and 4042 bp respectively (Plate 2). Amplified fragment of 16s rDNA with a gene size of 1555 bp was sequenced and analysed in genetic analyser 3130XL. Partial sequences of 23s rDNA and rpoB genes were obtained and the full length sequence of genes will be generated using sequencing primers by

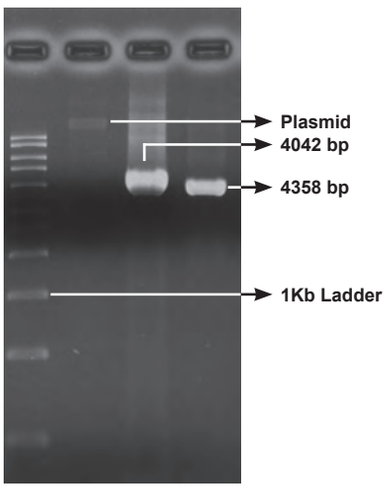
primer walking technique and analysed to see if VCRC B-17 could be differentiated from other Bti strains. Amplified fragments of endo- toxin genes also will be sequenced, to design VCRC B-17 specific probe for the quantification of crystal toxin by real time PCR.

ELISA for quantification of crystal toxin: Six batches of *Bti* were produced and crystals were separated and purified for raising antibodies to develop an ELISA technique for the quantification of crystal toxin (Plate 3).



Lane 1: 16s rDNA Lane 3: 23s rDNA
Lane 2: rpoB Lane 4: 1 KB Ladder

Plate 1 Amplicons of taxonomic marker genes



Lane 1: 1 Kb Ladder Lane 3: Cry4Aa
Lane 2: Plasmid Lane 4: Cry4Ba

Plate 2 Amplicons of endo-toxin genes

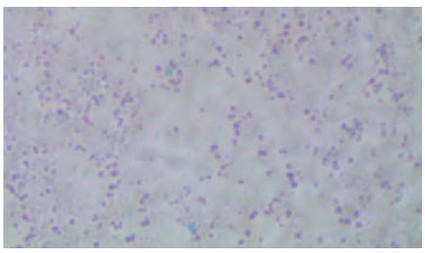


Plate 3 *Bti* Crystal toxin (100 X)

1.4.5. 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

IM1302: Apr 2013 – Mar 2016

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

An indigenous strain of *Bacillus amyloliquefaciens* (VCRC B483) was found to produce mosquitocidal and antibacterial metabolites. The production process was optimized by optimizing culture medium and downstream process. Antibacterial activity of the crude metabolite(s) was tested against clinical isolates and highest activity was observed with *Staphylococcus aureus*. Bacillomycin (bmy C), an antimicrobial metabolite encoding gene of 875 bp was amplified and sequenced (Annual Report 2013 & 2014).

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.

During the reporting year, using the optimized medium and downstream processes, 15 batches (1 lit/batch) were run and the yield of the crude metabolite obtained was 1.9 ± 0.4 g/lit. The metabolite yield in the conventional medium (NYSM) was found to be 0.8 ± 0.3 g/l. The efficacy of the crude metabolite(s) obtained from both the production media were tested against the pupal stages of *An. stephensi*, *Cx. quinquefasciatus* and A*e. aegypti* (Figure 1.25).

PCR conditions were standardized for amplification of all the antimicrobial peptide genes reported from this bacterium viz., Iturin, Bacilysin, Macrolactin, Bacillaene and Difficidin (Plate 4). Antibacterial activity of the crude metabolite(s) was tested against clinical isolates of methicillin Resistant *Staphylococcus aureus* (MRSA) (n=30) and Vancomycin resistant *Enterococcus* (VRE) (n=30). All the tested clinical isolates were sensitive to the Crude metabolites and showed zones of size ranging from 15–30 mm. A clinical isolate of *Streptococcus pneumoniae* tested was also found to be sensitive to the metabolite(s) (zone size 26 mm).

The crude metabolites of *B. amyloliquefaciens* contain a variety of peptides and the peptide

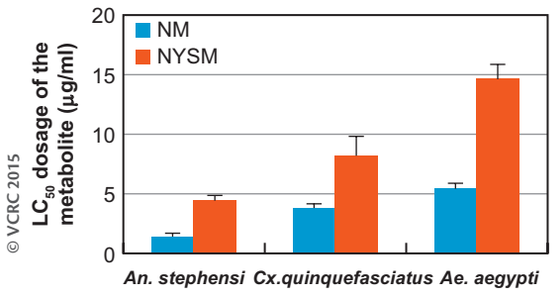
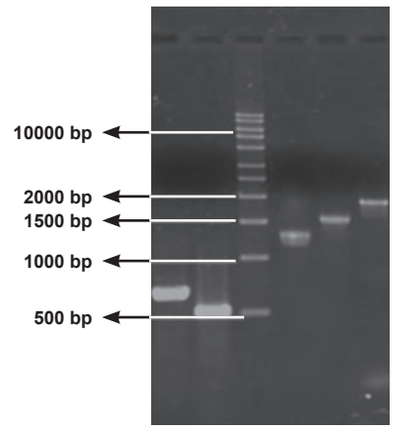


Figure 1.25 Efficacy of metabolites of *B. amyloliquefaciens* (VCRC B483) against pupae of major mosquito vectors.



Lane 1: Iturin
Lane 2: Bacilysin
Lane 3: 1 KB DNA ladder
Lane 4: Macrolactin
Lane 5: Bacillaene
Lane 6: Difficidin

Plate 4 PCR amplification of antimicrobial metabolite encoding genes of *B. amyloliquefaciens* (VCRC B-483)

responsible for the antimicrobial activity needs to be identified. Hence, the crude metabolites are being subjected to column chromatography for purification of the various peptides for further testing.

Sequencing of the antimicrobial peptide genes is to be carried out.

1.4.6. Development of naphthoquinone analogues as macrofilaricidal agent

IM 1306: Feb 2014 – Feb 2016

Nisha Mathew & Paily KP

Earlier, 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) with IFS funding. Subsequently for lead optimization, analogue molecules were synthesized with DST funding. 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 targeted substituted naphthoquinone analogues, the synthesis, *in vitro* macrofilaricidal activity screening, studies on chemical stability and aqueous solubility for compounds TR-NPQ1-6 were reported in the previous year (Annual Report, 2014). During the current year, the remaining five compounds TR-NPQ 7–11 have been 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 1.26. All the five compounds exhibited *in vitro* macrofilaricidal activity in the preliminary screening at 0.1mg/ml.

Poor aqueous solubility and poor dissolution in the GI fluids could be limiting factors for the *in vivo* bioavailability of the drug after oral administration. Drug solubility of the lead molecules was studied in comparison with standard drugs of high and low solubility (Figure 1.27). 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 for TR-NPQ 7, 8, 9 & 11 obtained in our

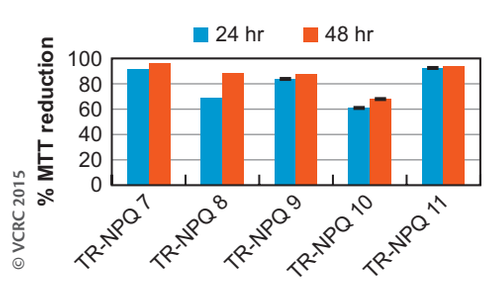


Figure 1.26 *In vitro* macrofilaricidal activity of synthesized TR-NPQ 1-11.

experiments are within the range of the reported values.

The results of the stability studies are given in Figure 1.28. The stability study in PBS pH 7.4, SGF and SIF revealed that the compounds TR-NPQ 8 & 9 are stable at both conditions indicating its acceptability for intended absorption from the high surface area of the intestine. In the case of TR-NPQ7, 10 & 11 the stability at pH 7.4 after 24 hrs was slightly reduced. However, with SIF and SGF it was found to be stable except for 11.

Studies on permeability in comparison to three standard compounds of different permeability (high: Propranolol HCl, carbamazepine; medium: Warfarin) showed that TR-NPQ 1, 2, 4, 5, 6, 7, 10 & 11 are highly permeable while TR-NPQ 3, 8 & 9 are with moderate permeability. For TR-NPQ 1, 4, 5 & 7 (Log D = 0.25–2.64) falls in the moderate Log D group and, therefore, bears moderate permeability as well as solubility, whereas for TR-NPQ 2, 3 & 6 Log D was slightly higher (3.1–3.12) and for 8, 9, 10 & 11 Log D was found to be < 0 (Log D = -0.95–-0.51) indicating slightly low permeability (Figure 1.29 & 1.30). The Log D in cyclohexane/PBS ranged from 0.15 to 1.99 for TR-NPQ 1-7 and for TR-NPQ 1–7 and for 8, 9, 10 & 11 it ranged between -1.27–-0.44 (Figure 1.31).

The results of plasma protein binding for TR-NPQ 1–11 along with the standard drug showed that for TR-NPQ 1–11 the percentage binding to plasma protein was found to vary between 21.3 %–96.19%. In other words TR-NPQ 1–3 and 5–10 showed <80% binding (poorly binding) and TR-NPQ 4 is moderately binding (80-95%) and 11 showed high binding (>95%)(Figure 1.32). The present study showed that 59.96–97.95% of the

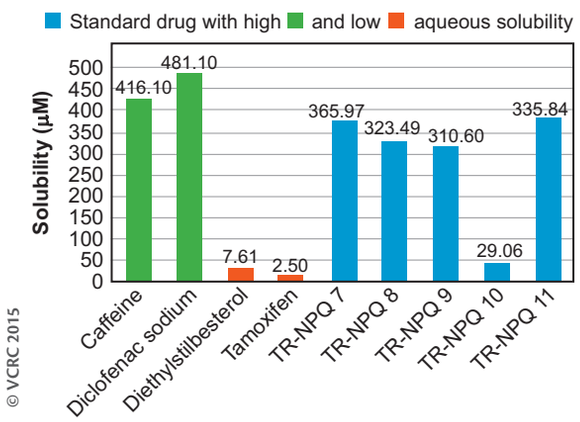


Figure 1.27 Solubility of TR-NPQ 7-11 and standard drugs in Phosphate buffered saline, pH 7.4.

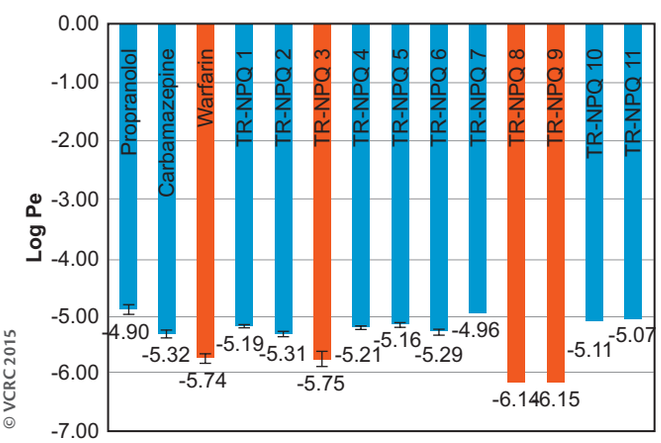


Figure 1.29 Log Pe values by PAMPA assay for TR-NPQ 1-11.

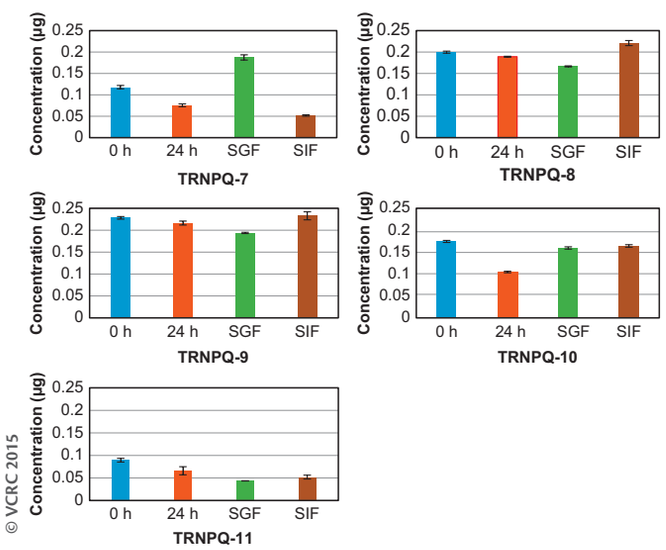


Figure 1.28 Chemical stability of TR-NPQ 7-11 in PBS pH 7.4, SGF and SIF.

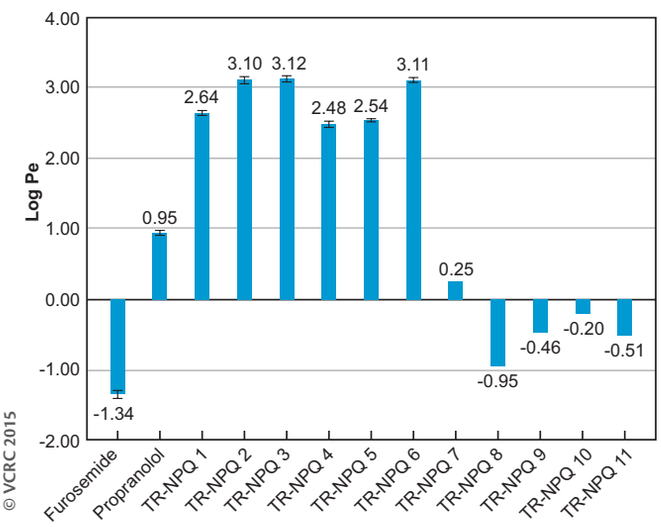


Figure 1.30 Distribution coefficient (Log D) values by Octanol/PBS partition for TR-NPQ 1-11.

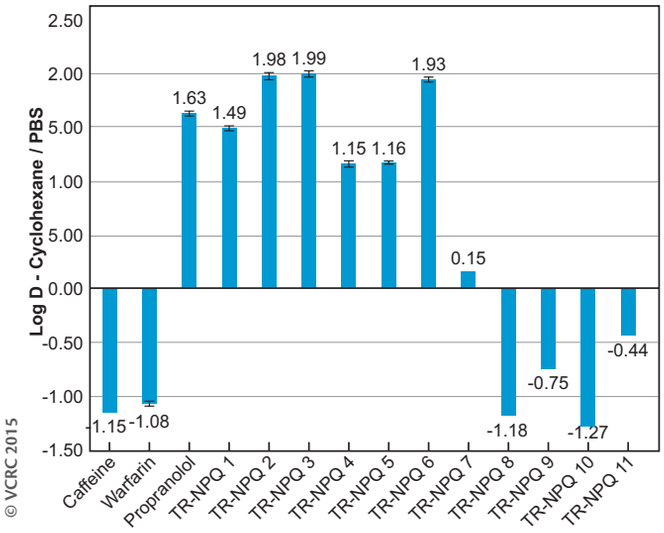


Figure 1.31 Distribution coefficient (Log D) values by Cyclohexane/ PBS partition for TR-NPQ 1-11.

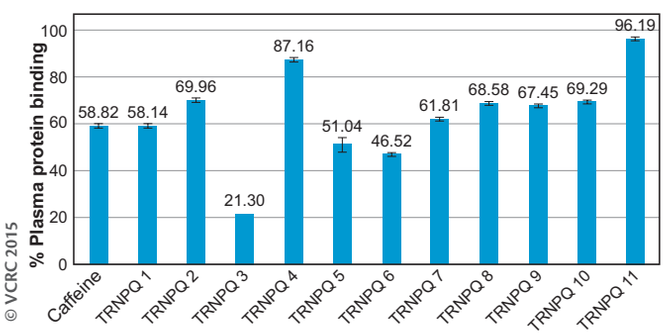


Figure 1.32 Percentage of plasma protein binding to TR-NPQ 1-11.

test compounds are remaining after metabolism (Figure 1.33), which indicates acceptable metabolic stability as well as indication of hepatic clearance.

Thus all the targeted 11 compounds have been synthesized, purified by column chromatography, purity checked by TLC and HPLC, analysed the chemical structures by FT-IR, ^{1}HNMR and ^{13}CNMR spectra, screened for *in vitro* macrofilaricidal activity and studied the *in vitro* ADME properties in comparison with standard drugs. Based on the ADME results six compounds *viz*., TR-NPQ 1, 2, 4-7 needs to be evaluated for *in vivo* macrofilaricidal activity. These compounds have to be sent to CDRI, Lucknow to get it evaluated because of the limited facilities at VCRC.

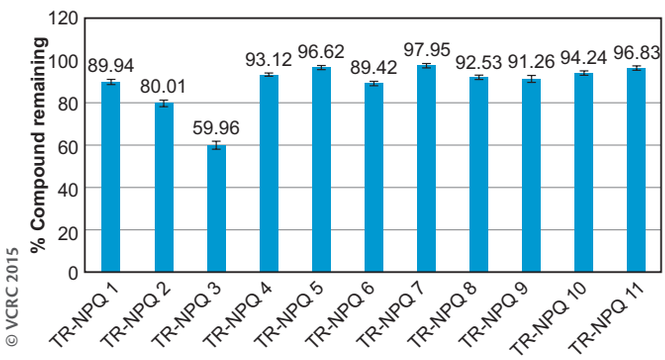


Figure 1.33 Metabolism of TR-NPQ 1-11 by rat liver Microsomal enzymes.

1.5 NEW VECTOR CONTROL TOOLS

Evaluation of MAGNet, an alpha-cypermethrin long-lasting insecticidal mosquito net, against susceptible malaria vector populations in experimental huts in Odisha State, India

EM 1507: Jun 2015 – May 2016

Gunasekaran K, Sahu SS, Vijayakumar T, Md. Mustafa Baig & Subramanian S

Use of long lasting insecticidal nets (LLINs), which do not require re-treatment throughout their expected life span of about three years or even more, is an important component in malaria vector control. MAGNet is one such LLIN, complied with the WHO specifications (454/LN/2; October 2009) with reference to total content of alpha-cypermethrin, the synthetic pyrethroid used for the treatment, and retention index. The cone bioassays on MAGNet showed a mosquito knock down (KD) of >95% and/or mortality of >80% for 20 washes, thereby the LLIN met the WHO criteria for the Phase I study (WHO/HTM/NTD/WHOPES/2011.7). The efficacy of MAGNet is evaluated in experimental huts (Phase II evaluation) against the susceptible population of *Anopheles fluviatilis* sensu lato in terms of mortality, deterrence, blood-feeding inhibition and induced exophily in Odisha State, following the ICMR's Common Protocol.

Objectives:

General:

- To determine the efficacy of MAGNet washed 20/25 times relative to the WHO recommended reference LLIN, Duranet (Specification No.: 454/LN/2), washed 20 times against susceptible *An. fluviatilis* mosquitoes in experimental huts.

Specific:

- To study the effectiveness of MAGNet in terms of causing immediate & delayed vector mortality.
- To assess the effectiveness of MAGNet on density and behaviour of the vector(s), i.e. reduction of entry rate, feeding success (engorging rate) & excito-repellency (comparison of exit to entry rate).
- To determine the efficacy of washed and unwashed LNs against malaria vector(s).

The evaluation includes nets of six arms viz., 1) Unwashed MAGNet, 2) MAGNet washed 20 times, 3) Unwashed Duranet, 4) Duranet washed 20 times, 5) Untreated net and 6) MAGNet washed 25 times. There are six replicate nets in each arm, to be used in six experimental huts on rotation. Each arm has also two additional nets for conducting bioassay and chemical analysis prior to any wash and after washing. The candidate LLIN (MAGNet) and nets of the other five arms were received from the sponsor. All nets were coded to indicate the arm and the replicate nets of each arm. Washing of nets has been initiated and so far nets were washed 13-16 times; washing is continued.

The cone-bioassay conducted prior to any wash on the designated net of each arm showed 100% mortality of *An. stephensi* with all arms, except one, might be the untreated net, with which the mortality was only 0%. Testing for the suitability of the six experimental huts constructed at Kandhaguda village of Malkangiri district in Odisha State has been started. Vector density in comparison to village huts, recovery rate of released mosquitoes and absence of scavenging inside the huts are the parameters considered to assess hut suitability. Once washing of nets is completed, they will be evaluated in experimental huts for their effects on free-flying, wild *An. fluviatilis* mosquitoes and for their ability to deter entry, repel or drive mosquitoes out of houses, induce mortality and inhibit blood-feeding.

1.6 BIOMEDICAL INFORMATICS

Biomedical Informatics Centre

EM: Jan 2014 – Dec 2018

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

Biomedical informatics centre (BMIC) is established at Vector Control Research Centre in January 2014. This centre is involved in the collaborative and multi disciplinary research works of the institute. The main target of the BMIC is the development of a database of Indian vectors with full fledged annotation of each disease causing vector. In addition to the database, the centre also works on the insecticide resistance mechanism, diagnostic tools and drug discovery strategies. The centre has already initiated and established a stable platform in these fields and the research work is in progress with fruitful results.

Objectives:

- The BMIC has been started with the first four objectives (given below) in the year 2014. The objectives No: 5 & 6 have been added in 2015.
- To study prevalence of arbo-viruses in India with reference to their genomics, meta-genomics and molecular adaptation to vectors.
- To verify the role of kdr mutations in Voltage Gated Sodium Channel (VGSC) in conferring target mediated resistance to DDT and synthetic pyrethroids in malaria vectors.
- To detect and identify antigenic determinants of proteins of *Wuchereria bancrofti*.
- Identification of lead compounds as mosquito attractants/repellents.
- Development of software for DNA-Barcode analysis – a tool for identification of mosquito species.

Objective 1: Development of vector informatics database (VectorInfo)

VectorInfo: A first ever repository of medically important Indian arthropods has been initiated and designed to offer information ranging from basic biology, molecular aspects to control strategies of vectors that are known to pose serious threat to humans and livestock. Development of VectorInfo will be accomplished through three phases of activity (Figure 1.34).

Phase I: Website development - It is a compilation of data on the established, emerging and

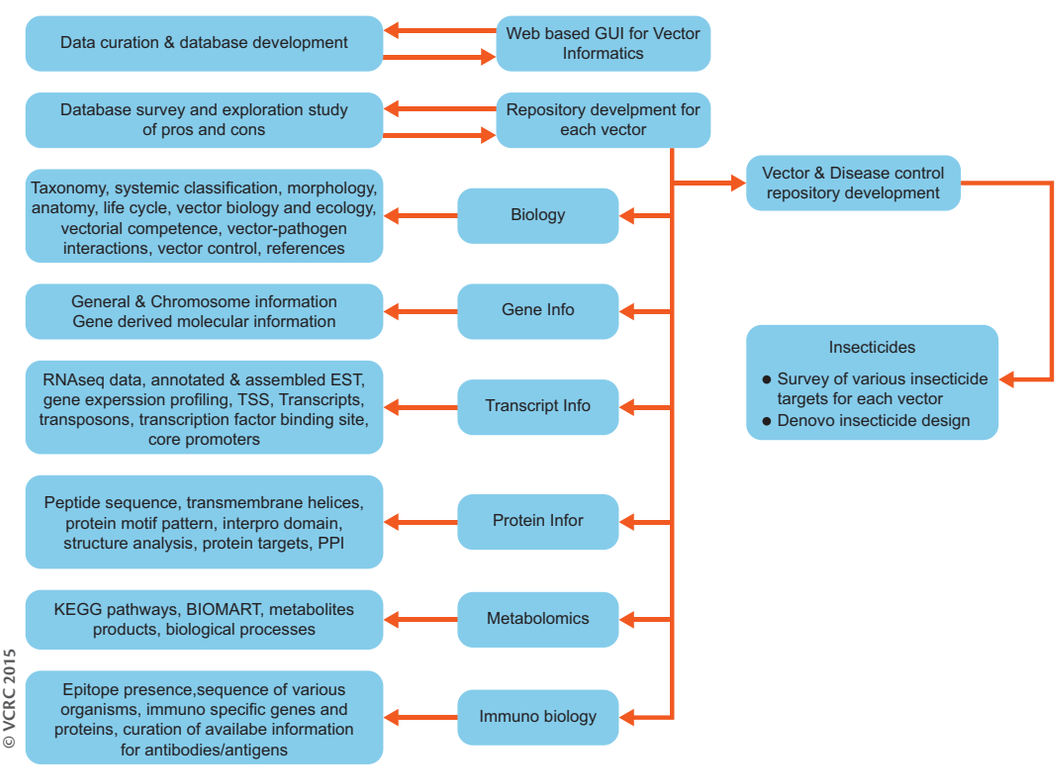


Figure 1.34 The schematic plan of VectorInfo database.

re-emerging vector borne diseases of anthroponotic and zoonotic origin with specific reference to Indian scenario. **Phase II:** VectorInfo database - The nitty-gritty of this database is aimed at scrutinizing all the possible information on Indian disease vectors in a single window and user friendly. **Phase III:** Insecticides and their targets - identifying validated insecticide targets and their mechanism paves way to design and develop novel lead molecules through computational means.

Phase I has been completed and is under internal validation. **Phase II** is in progress. **Phase III** is to be initiated.

Objective 2: To study the prevalence of arbo-viruses in India with reference to their genomics, metagenomics and molecular adaptation to vectors

Arbo-viral diseases are on an emerging trend across the globe and whole genome analysis of strains/serotypes/genotypes etc. would provide an insight into the increasing incidence of these diseases. VCRC has been maintaining a depository of about 200 isolates of arbo-viruses belonging to different strains/serotypes/genotypes since 2006. Studies are being initiated to analyse the genome of DENV, so as to have an understanding of the evolutionary trend of viruses and its implications on the increased virulence and incidence of these diseases.

A unique mutation was recorded in the Domain II of the Envelope gene (EDII) of the DENV-3 genome at the amino acid position 219 (A219T).

The evolutionary implication of this non-synonymous mutation near the EDI/EDII hinge is being investigated. Further the impact of the recorded mutation is to be analysed through molecular dynamics simulations.

Objective 3: To verify the role of *kdr* mutations in Voltage Gated Sodium Channel (VGSC) in conferring target mediated resistance to DDT and synthetic pyrethroids in malaria vectors

Due to the extensive use of insecticides in vector control programme, there has been development of resistance in vector species such as knockdown resistance (*kdr*). Insects exhibiting *kdr* have reduced target-site (sodium channel) sensitivity to pyrethroids and DDT resulting from one or more point mutations in the insect sodium channel protein.

The objective is to study the molecular mechanism related to insecticidal resistance arising due to point mutations by generating mutant models based on the wild type model of VGSC.

The sodium transport domain of VGSC was constructed by de novo modelling and subjected to molecular dynamics simulations. The low energy confirmation was taken as wild type models and

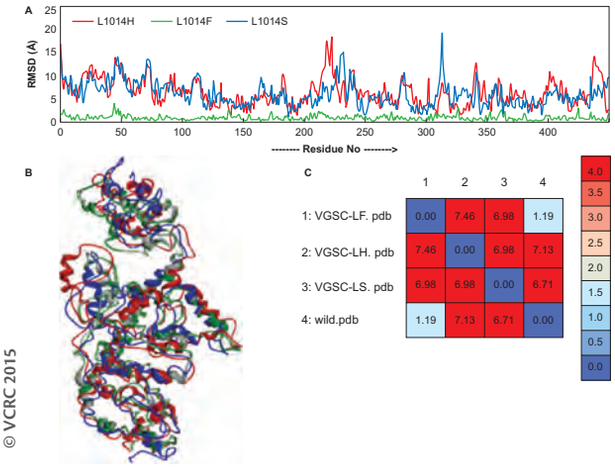


Figure 1.35 (A) Residue-RMSD plot of mutated structures; (B) Superimposed structures of wild type and mutant VGSC sodium transport domains; (C) RMSD matrix explaining the RMSD variations among the wild type and mutant VGSC sodium transport domains.

from this, L1014F, L1014S, L1014H mutant models were constructed. These mutant models were also subjected to molecular dynamics simulations and the variation in energy levels and conformations were studied (Figure 1.35).

These molecular dynamics simulated wild type and mutant models will be used to identify the binding mode variations of DDT and synthetic pyrethroids through molecular docking studies. All these analyses finally will give the plausible explanations for the insecticide resistance mechanism due to mutations.

Objective 4: To detect and identify antigenic determinants of proteins of *Wuchereria bancrofti*

Lymphatic Filariasis (LF) caused by *W. bancrofti* is a severe incapacitating, non-noxious disease of tropical and sub-tropical regions. Post MDA (Mass Drug Administration) monitoring certification is based on ICT (Immuno Chromatographic Test cards), which is imported and expensive, besides issues on the size of the evaluation unit it should encompass. Hence we aimed to develop an indigenous antigen based diagnostic tool to meet the urgent demand.

In this context we have considered three proteins viz., cuticular collagen (CC), Alt3 and Enolase from *W. bancrofti* for the detection and evaluation of antigenic determinants. Initially potential antigenic determinants were identified in CC and located in the *de novo* model to check their availability on the surface of the structure. The predicted determinants were checked with all antigenic criteria and finally four peptides were identified in CC.

These peptides have been synthesized and are under evaluation for their immunogenicity through *in vitro* means.

Based on the results obtained from *in vitro* assays further optimizations will be made in the antigenic peptides so as to enhance their immunogenicity.

Objective 5: Identification of lead compounds as mosquito attractants/repellents

Chemical attractants and or repellents are the most potential insect control agents that are required to meet the demands of public health agencies. These compounds have the ability to stimulate the movement of mosquitoes towards or away from the compound. In this context, we aimed to identify leads that could be developed as attractants or repellents by testing against specific proteins like odorant receptor and odorant binding proteins of mosquitoes through *in silico* means. These computational modelling studies potentially provides the basic idea of attractance or repellence capacity of specific compounds based on its inter-molecular interactions with the targets.

As an initial investigation, we presently have chosen the odorant receptor protein (OR4) from *Aedes aegypti* to find the molecular mechanism of several lead compounds that were collected from earlier reports and known to be present as human skin surfactants.

The three dimensional model of OR4 was constructed and its stereo chemical quality was validated. The structure was energy minimized and subjected to molecular dynamics simulations and the low energy conformation was chosen for molecular docking studies. A total of 354 leads were collected and docked against the predicted binding site of OR4. The protein-ligand complexes were evaluated based on the Libdock scores and their intermolecular interactions were analysed.

Some interesting results were observed from molecular docking studies where the compounds were specifically binding at three different locations in the OR4 although a same binding sphere was defined during molecular docking studies (Figure 1.36).

This provides a critical basis of their interaction that will give an idea to cluster them, based on the site of interactions, as attractants, oviposition attractants and repellents. Further the information about the intermolecular interactions will be used to develop potential attractants/repellents.

Objective 6: Development of software for DNA-Barcode analysis – a tool for identification of mosquito species

The aim of DNA-Barcode software is to provide an efficient method for the identification of genus/species by using the mitochondrial Cytochrome-C oxidase subunit 1 (COI) sequences to reduce the species identification ambiguity. COI sequences of Vector Control Research Centre (VCRC) were taken for the training set and the COI from NCBI data base were considered as test set. The number of sequences varies depends on the availability as species wise.

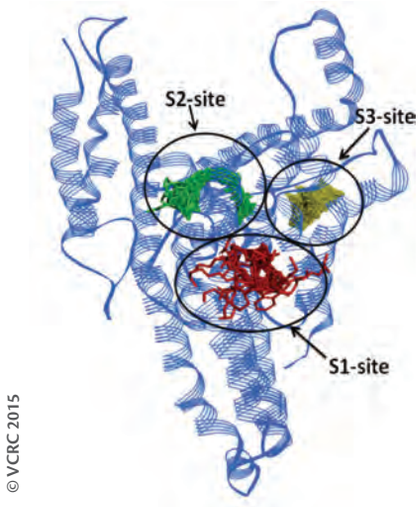


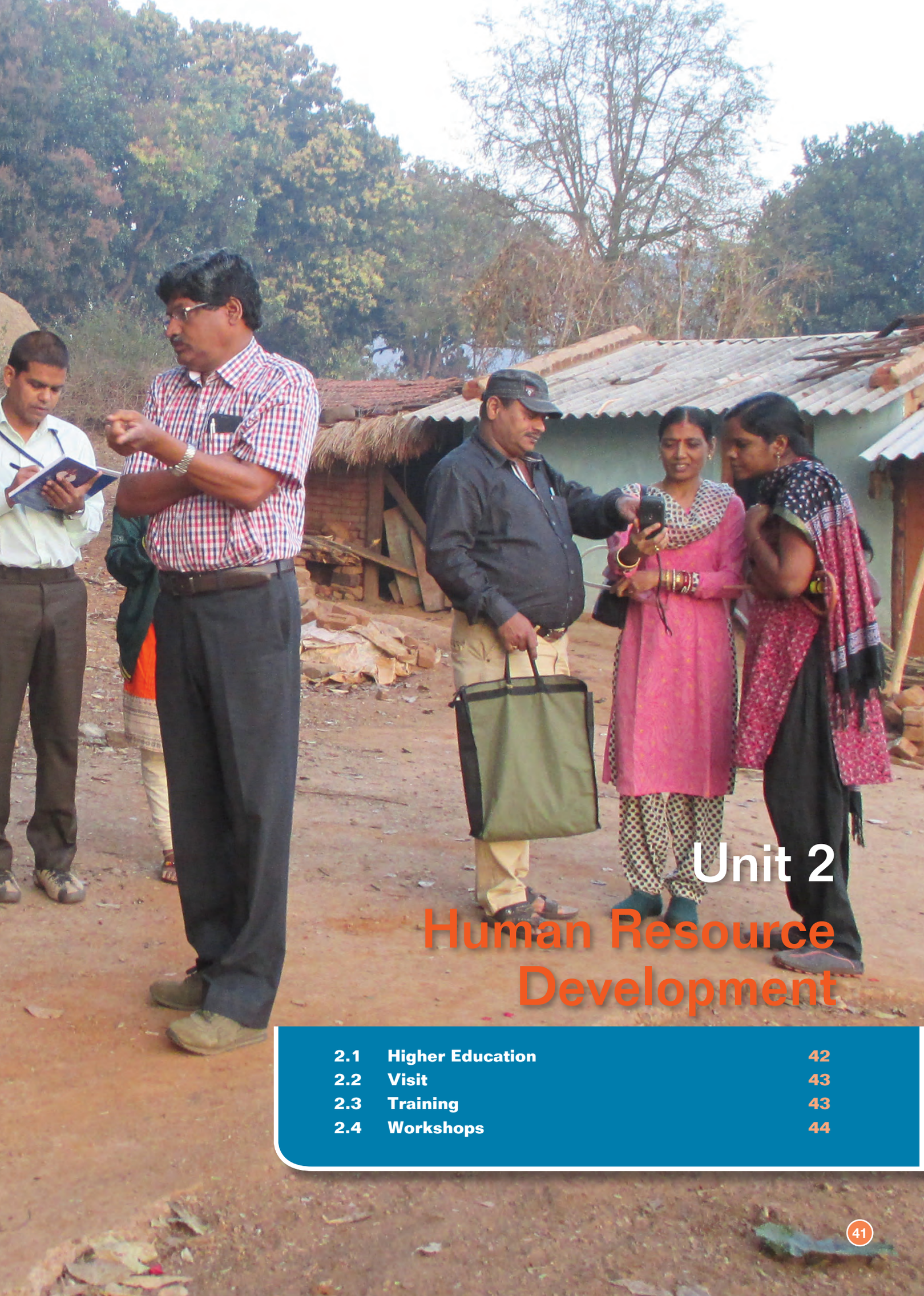
Figure 1.36 Three dimensional structure of OR4 from *Aedes aegypti* with three binding sites S1, S2 and S3 wher the ligands have specific binding.

We aimed to develop software using JAVA programming language to create barcode image and also to identify the unique repeated pattern which are species and genus specific. The tool provides the facility to examine the presence of patterns in the sequence. The output of barcode image and repeated patterns are stored as the image and text files respectively. The input COI sequence can be uploaded as a text file or directly copy and paste from the source. The generated outputs are maintained in an excel file for database uploading and pattern comparison. Further, species and genus wise repeated patterns will be identified for training and test sets. Patterns which are common in both sets will be considered as final and stored in the database.

Finally web based interface will be designed to provide these details of known species. User can view the data and download the patterns list and the barcode image from the web site.

So far the tool for barcode generation is developed and unique patterns were identified for *Aedes aegypti* and *Aedes albopictus* based on the training and test set data formed based on the available COI sequences from Indian mosquitoes.

Another software will also be developed and incorporated into the interface to identify the taxonomy of unknown species by comparing the existing genus/species repeated patterns from the database.



Unit 2
Human Resource Development

2.1 HIGHER EDUCATION

In view of the emerging and re-emerging vector-borne diseases in India and other tropical countries, there is a growing need for entomologists in the field of Public Health. In order to meet this demand, a two year M.Sc. Public Health Entomology (PHE) course & Ph.D. programmes (affiliated to Pondicherry University) are being conducted. Besides, a need based formal and informal training programmes for students and public health personnel are being offered.

2.1.1. M.Sc. Public Health Entomology

Twelve candidates have been admitted for the year 2015–17, fifth 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 is an in-service candidate (Multipurpose Health Supervisor) from Regional Director Medical & Health Services, Warangal, Telangana district.

From the M.Sc. PHE batch 2013–15, eight students have successfully completed the course and among them, four students have been selected for internship based on the inter-se merit list obtained from Pondicherry University.

As a part of the course curriculum, M.Sc., students visited Institutes such as National Centre for Disease Control, National Vector Borne Disease Control Programme, National Institute of Malaria Research in New Delhi, Rajendra Memorial Research Institute in Patna and National Centre for Disease Control in Bangalore. They acquired good knowledge on Dengue vectors, insecticide resistance, strategies in controlling major vector borne diseases, Sandflies identification, collection and its control methods and rodent surveillance during the above visits. Also they were taken to VCRC field stations at Koraput for Malaria study and Kottayam for Dengue and Chikungunya study.

2.1.2. Ph.D. Programmes

Seventeen full time (Zoology – 10; Microbiology – 5; Chemistry – 2) and three part time Internal (Zoology – 2 and Microbiology – 1) candidates continue to pursue their Ph.D. programme.

2.2 STUDENT'S VISIT

Visitors from different Institutes of India visited VCRC for orientation and exposure to various ongoing programmes of the centre.

S.No.	Name of the College / Institution	Course / Title	No. of Students
1	Lal Bahadur Shastri National Academy of Administration, Mussoorie	IAS Trainees	15
2	Pondicherry University Community College, Puducherry	Diploma in Sanitary Inspectors	50
3	Indira Gandhi Medical College & Research Institute, Puducherry	MBBS	45
4	St. Joseph Arts and Science College, Cuddalore	M.Sc.	33
5	Auroville Health Centre, Puducherry	Health workers	10
6	Indira Gandhi Medical College & Research Institute, Puducherry	MBBS	40
7	Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry	MBBS	72
8	Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry	B.Sc. Nursing	67

2.3 TRAINING

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

S.No.	Trainee particulars	No. of Trainees	Field of Training	Period
1	MD Students, Dept. of Community Medicine, Sri Manakula Vinayagar Medical College and Hospital, Puducherry	2	Methods of Vector Control	09.03.2015
2	MD Students, Dept. of Community Medicine, Rajah Muthaiah Medical College, Annamalai University, Chidambaram	8	Integrated Vector Control programmes	06.05.2015
3	M.Sc. Community Health Nursing College of Nursing, JIPMER, Puducherry	5	Filariasis	01.06.2015 to 03.06.2015
4	MD Students, Dept. of Community Medicine, Pondicherry Institute of Medical Sciences, Puducherry	3	Integrated Vector Management, Surveillance system for Malaria & Filariasis and GIS mapping of VBDs	Jun 2015 (1 week)
5	MD Students, Dept. of Community Medicine, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry	3	Methods of Vector Control	27.07.2015 to 29.07.2015

2.3.1. WHO Training Programmes

World Health Organization (WHO) has sponsored Medical Officers and Entomologists from DPR, Korea and Sri Lanka for training in the following areas:

S.No.	Trainee particulars	No. of Trainees	Field of Training	Period
1	Medical Officer, Central Hygeinic & Anti Epidemic Institute, Ministry of Public Health, DPR Korea	3	Malaria Entomology & Vector Control	Aug 2015 (4 weeks)
2	Medical Officer, Anti FilariasisCampaign, Colombo, Sri Lanka	4	Identification of vectors, IVC methods	Dec 2015 (2 Weeks)

2.3.2. Project work for Students from Foreign University

S.No.	Name of the Student	Institution	Period (weeks)	Subject/Title
1	Ms. Rohini Sanjeev Swamy (Post Graduate)	Department of International Health, Georgetown University, USA	9	Assessing the risk for dengue in an endemic village in Kerala, India
2	Ms. Sabiha Robia Hussain (Under Graduate)	Department of International Health, Georgetown University, USA	12	An evaluation of urbanization and health statistics in Puducherry with a special reference to dengue
3	Ms. Nina Lee Eng (Under Graduate)	Department of International Health, Georgetown University, USA	15	An environmental and entomological risk assessment of scrub typhus in rural villages in Puducherry
4	Ms. Leah Plasek	St. Olaf College, USA	6	Population genetic analysis of dengue vectors
5	Ms. Ambele Judith Mwamelo	St. Olaf College, USA	6	Socio-economic risk factors on the Endemicity of Dengue in foothills of Western Ghats, Kerala, India

2.3.3. Training programme at VCRC Field Station, Koraput, Odisha

Date	Training Details	Imparted By
12 Feb	Training to 20 TRW school Teachers of Koraput district at CDMO Conference Hall, Koraput on 'Use of RDK, blood smear collectionfor malaria diagnosis and treatment' organized jointly by the District Health and Social Welfare Departments, Koraput	Dr. S.S. Sahu and the District Health Officials, Koraput
25 Feb	Training to 23 Field workers of Laxmipur and Dasmantapur CHCs of Koraput district at VCRC Field Station, Koraput on ''Use of RDK, blood smear collection formalaria diagnosis and treatment'' organized by VCRC FS, Koraput	Dr. S.S. Sahu
18–27 Mar	Training to 86 Health Workers of Laxmipur, Dasamantapur, N. Patana and Bandhugaon CHCs of Koraput district at CDMO Conference Hall, Koraput on 'Role of Health Workers (male and female) on control of malaria and other vector borne diseases' organised by the Department of Health, Bhubaneswar	Dr. S.S. Sahu and the Health Department Officials, Bhubaneswar
26 May–4 Jun	Training to Assistant Entomologists of Govt. of Odisha at VCRC Field Station, Koraput on 'Malaria Entomology and vector control methods' organised by the Health Department, Bhubaneswar	Dr. S.S. Sahu

2.4 WORKSHOPS

2.4.1. Regional Training of Trainers' (ToT) Workshop on Transmission Assessment Survey in Lymphatic Filariasis, Vector Control Research Centre, Puducherry, India 23–26 June 2015

The NTD-STAG 2015 recommends the use of Filariasis Test Strip as the diagnostic test of choice for *W. bancrofti* in the programme without changes to the current TAS guidelines. Further, in view of integration of NTDs targeted for elimination using under preventive chemotherapy approach, there is a need to integrate assessment of STH with TAS so as to assist the programme to assess the prevalence of STH to decide on the frequency of mass treatment for STH control. The Vector Control Research Centre (VCRC) and WHO-SEARO jointly organized a capacity building workshop in Puducherry, 23–26 June 2015. The objective of the training was to update the progress of the national LF elimination programme in each country, impart knowledge and enhance skills of the LF programme managers or focal points on planning and implementation of TAS and develop long term action plans. There were 26 participants from seven countries, including four focal points and 2 from RTI. The group was a heterogeneous, ranging from programme managers to laboratory technicians.

This training workshop was based on 11 modules including the new STH integration module. The faculty included experts in the respective field from WHO, VCRC, Puducherry and CDC Atlanta. The pre-test and post-test were designed to assess the areas for more attention and the improvement in knowledge and skills of the participants after completing training. Participants' feedback on the contents of the training and training methodologies showed that the satisfaction index is above 80%. The participant's pre-test knowledge score was 53.7% and the post-test score was 78.3% with a positive shift of 1.46 times. The feedback and knowledge scores indicated that workshop was highly productive. Each participant prepared TAS plan for an EU and the participants from the member countries developed long term action plan, which is an important outcome of the exercise for forecasting diagnostic kit requirements.

2.4.2. National Training of Trainers' (ToT) Workshop on Transmission Assessment Survey in Lymphatic Filariasis, Vector Control Research Centre, Puducherry, India 14–17 July 2015

A National Training of Trainers' (ToT) Workshop on Transmission Assessment Survey in Lymphatic Filariasis was also conducted by Vector Control Research Centre, Puducherry and the WHO Country Office, New Delhi, 14–17 July 2015. This workshop is a follow-up of a series of training programmes conducted during 2013 in four different regions in India with the support of WHO Country Office.

The objective of the training was to update the progress of the national LF elimination programme in the country, develop skill on the use of the FST in assessing the incidence of filarial infection using the revised TAS protocol, develop action plan for implementing TAS for stopping MDA and monitoring during post MDA period and acquire the knowledge to enable integration of STH assessment along with the TAS impart knowledge and enhance skills of the LF programme managers. There were 29 participants from the endemic states and union territories and the group was a heterogeneous, ranging from Senior Regional Directors to Filaria Officers.

Eleven modules were used for the training. There were small group exercises and on completion of all the modules, each participant prepared a TAS plan for an evaluation unit. A new STH integration module was included. The faculty included experts in the respective field from WHO, NVBDCP and VCRC. Tests prior to and after training were conducted to assess the impact of training. The participant's pre-test knowledge score was 47.58% and the post-test score was 76.0% with a positive shift of 1.6 times. Feedback from the participants were also received on the contents of the module and areas that require more attention in the training. Satisfaction index was above 80%. The feedback and knowledge scores indicated that workshop was highly productive. At the end of the training each participant prepared TAS plan for an EU in the respective area their work.

Unit 3
Technical Support

3.1 SERVICE AND SUPPLIES

3.1.1. 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

Vector susceptibility tests are conducted in field using WHO tubes and insecticide impregnated papers (IIPs) at the diagnostic concentrations. Currently, the IIPs are imported from Malaysia. The NVBDCP often come across difficulties in procurement of papers resulting delay in delivering these papers to different regions. Hence, this project was initiated by ICMR on the request of NVBDCP to establish a facility at VCRC, Puducherry to prepare the IIPs indigenously.

Objective:

- 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.

VCRC has standardized the procedure for the preparation of IIPs of 12x15cm size Whatman no.1 filter papers with Technical grade organochlorine insecticide DDT using Risella oil as the base. For organophosphorus insecticide malathion, Olive oil was used as the base. For synthetic pyrethroids like, Deltamethrin, Alpha-Cypermethrin and Lambda-Cyhalothrin, Silicone oil was used as the base. Respective control papers were made with the base oils alone without the insecticides.

Quality checking of the IIPs was done by insecticide content analysis by GC/MS at NHRDF, Nasik for 4% DDT, 5% Malathion and 0.05% Deltamethrin and reported (Annual Report 2014). The insecticide content analysis of 0.05% Alpha-Cypermethrin and 0.05% Lambda-Cyhalothrin impregnated papers were carried out by HPLC at Division of Chemistry, VCRC and by bioassay at Division of Vector Biology and Control, VCRC in comparison with WHO papers.

The comparison of Alpha-Cypermethrin content in impregnated papers (WHO and VCRC) is given in Figure 3.1.

The comparison of Lambda-Cyhalothrincontent in impregnated papers (WHO and VCRC) is given in Figure 3.2.

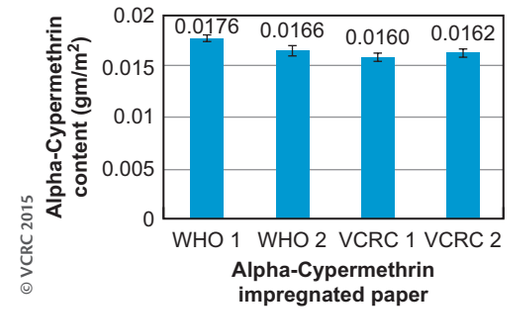


Figure 3.1 Alpha-Cypermethrin content analysis in WHO and VCRC IIPs by HPLC.

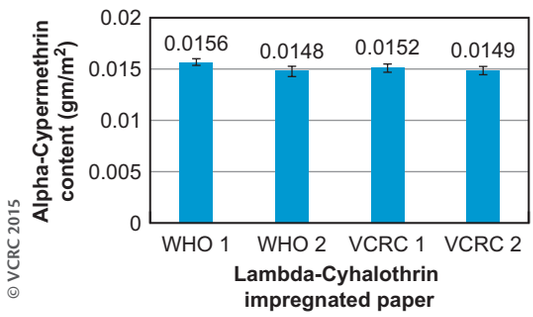


Figure 3.2 Lambda-Cyhalothrin content analysis in WHO and VCRC IIPs by HPLC.

The HPLC analysis shows that the quality of the insecticide impregnated papers were almost similar to that of WHO papers in terms of insecticide content in of 0.05% Alpha-Cypermethrin and 0.05% Lambda-Cyhalothrin impregnated papers. Bioassay results showed comparable results as that of WHO insecticide impregnated papers (Figure 3.3).

Insecticide impregnated papers *viz.*, 4% DDT, 5%Malathion, 0.05% Alpha cypermethrin, 0.05% Lambda Cyhalothrin, and 0.05% Deltamethrin were prepared and submitted for multicentric evaluation at two different institutes *viz.*, NIMR (ICMR), Delhi and Institute of Vector Control and Zoonoses, Hosur along with respective control papers at a meeting held at ICMR on 19th August, 2015. WHO papers also were supplied as positive control. Results are awaited.

Facilities will be established for supply of insecticide impregnated papers to NVBDCP as per their requirement.

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

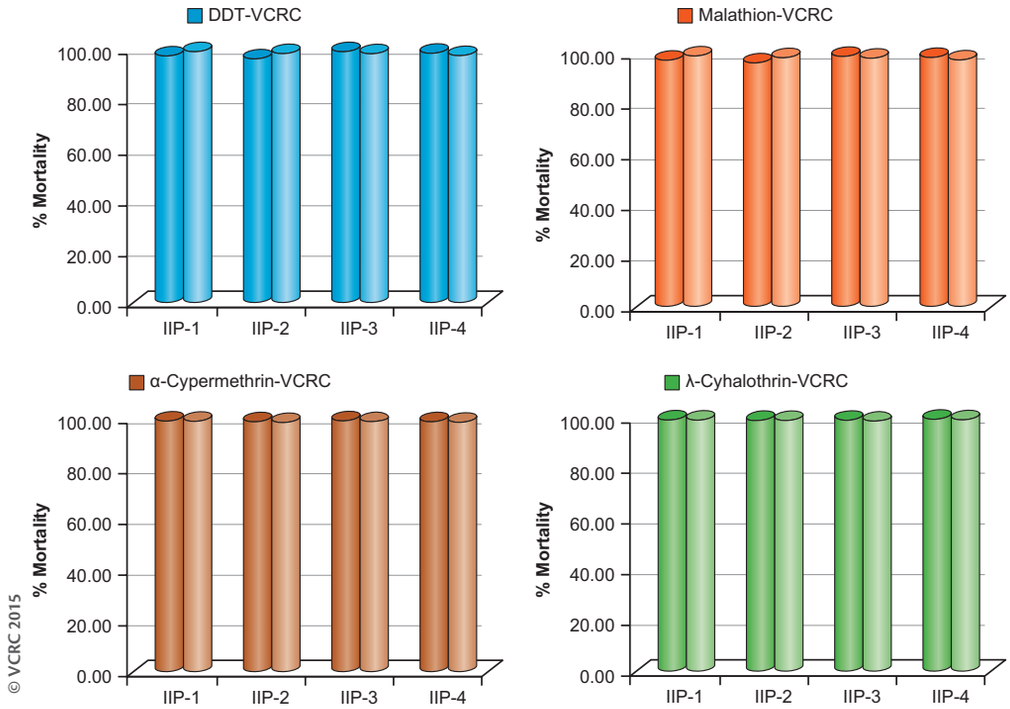


Figure 3.3 Susceptibility of adult *An. stephensi* to insecticide impregnated papers of VCRC in comparison with WHO papers.

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.

Objective:

- To assess the response of *An. culicifacies* to the insecticides under use.

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).

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

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

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. Susceptibility status of *An. fluviatilis* and *An. culicifacies*, the malaria vectors, to DDT and deltamethrin in Rayagada District of Odisha State, India

Sep 2015 – Nov 2015

Sahu SS & Gunasekaran K

Rayagada, one of the southern districts of Odisha State, has been severely affected by malaria. In 2014, >13% of the total positive cases recorded in the State were reported from the district alone. From 2010 onwards, malaria incidence was on an increasing trend in the district, with annual parasite incidence ranging from 50.0 to 54.2. *Anopheles fluviatilis* and *An. culicifacies* are the two malaria vectors prevalent in the district. Under malaria vector control programme of the NVBDCP, indoor residual spraying has been carried out with DDT in eight Community Health Centres (CHCs) and with deltamethrin in three CHCs. In addition, long lasting insecticidal (deltamethrin) nets (LLINs) have been distributed (during 2012) in the entire district. However, the desired impact has not been achieved from the intervention measures. Therefore, it is felt necessary to assess the susceptibility status of the two vector species to DDT and deltamethrin for rationalizing their use for vector control.

Objective:

- To determine the susceptibility of *An. fluviatilis* and *An. culicifacies* to DDT and deltamethrin.

Out of the total 11 CHCs in the district, two CHCs i.e. Bissum cuttack (DDT sprayed area) and Kolnara (SP sprayed area) were randomly selected for the study. In each CHC, five villages were randomly selected for collection of mosquitoes for susceptibility tests. Female mosquitoes were collected from cattle sheds and human dwellings of the selected villages. The tests were performed on wild-caught blood-fed *An. culicifacies* collected with mouth aspirators in the morning hours between 6 AM and 8 AM. Susceptibility test was performed as per the WHO susceptibility test procedure. According to the WHO criteria, if the corrected mortality of a mosquito species on exposure to the diagnostic dosage of a given insecticide is ≥98%, it is 'susceptible', 90-97% it is under 'verification required' and <90% it is 'resistant'. Testing *An. culicifacies* for its susceptibility to DDT (4%) and deltamethrin (0.05%) was completed (Table 3.2); this species was found resistant to both DDT and deltamethrin. Tests with *An. fluviatilis* will be conducted during the season of its abundance (post-rainy/ cooler months).

Table 3.2 Results of susceptibility tests with *An. culicifacies*

Name of the CHC	Insecticide	Number exposed		Number dead		Corrected Mortality (%)
		Treated	Control	Treated	Control	
Kolnara	DDT 4.0%	157	55	41	2	23.3
	Deltamethrin 0.05%	165	57	130	1	72.2
Bissam Cuttack	DDT 4.0%	157	55	34	1	20.2
	Deltamethrin 0.05%	190	61	154	1	80.8

3.2 EPIDEMIC INVESTIGATIONS

3.2.1. Investigation at Nagamalai, Karulai PHC, Malappuram district

In Mallapuram district of Kerala, 5 confirmed cases of KFD and 3 suspected KFD deaths were reported in Nagamalai hills of Nedumkayam reserve forest during December 2014. In addition, monkey deaths due to KFD were reported in Karulai, Amarambalam, Chaliyar and Chugathara villages of Nilambur Taluk, Malappuram district, Kerala during Jan–May 2015.

Following the report, case investigation and entomological survey were carried out during the first week of

May 2015. A team comprising of VCRC Scientists, District Surveillance Officer, Karulai PHC staff from Malappuram district along with Anti-terrorist squad commandos visited the Nagamalai hills on 4–5 May 2015 for investigation. As the area (Nagamalai) is a reserve forest and inhabited by the primitive tribes, permission of the District collector, Malappuram was obtained prior to the investigation.

The study area is a thick tall rain forest with a diverse wild life. The area is inhabited by the primitive Cholanayakan tribe live inside caves or on hill slopes in the forest and do not own any domestic animals. A total of 196 Cholanayakkans live in 17 families distributed in Nagamalai hills located at an altitude of 2900 feet MSL. They earn their livelihood by collecting honey and various forest produce and visit to nearby Nilambur town for exchanging their forest produce for food grains and other essential supplies.

At Nagamalai, case investigation was carried out with a 22 years old male and his father aged 65 years who had suffered from KFD/monkey fever. The son believed that he suffered from fever after trapping the monkeys. The Cholanayakkans have the habit of eating the meat of monkeys, particularly the black faced langurs. The area (Nagamalai hill) is with full of tall rain forest and semi deciduous forest that support large number of animal reservoir (natural hosts) of KFDV and also survival and multiplication of various species of *Haemaphysalis* ticks. As the Cholanayakkans live in deep and dense forest, they are continuously exposed to tick bites. Further, as they trap monkeys for eating, this tribe has very high risk of contracting the KFDV through infected tick bites while handling the trapped monkeys. Therefore, vaccination of all the Cholanayakkan tribe living in the Nagamalai hills should be carried out in order to save the primitive tribal community.

3.2.2. Investigation report of JE death in Puducherry, 2015 – Entomological risk assessment

Sri. V. Rithik, S/o. Mr. Vaithiyanathan, a 3 year 6 months old boy died on 1st August 2015 reportedly due to *Japanese encephalitis* (JE) at Keezh Sathamangalampet, Puducherry.

It has been reported that on 23rd June, 2015, the boy was bitten by a stray dog and he had undertaken treatment for the same at JIPMER Hospital, Puducherry. He was not well and suffering from fever for about 20 days and had taken treatment at Rajiv Gandhi Government Women and Children's Hospital, JIPMER and Indira Gandhi Government General Hospital at Puducherry. As the condition of boy was very much deteriorating, he was admitted at Institute of Child Health and Hospital for Children (ICHH), Egmore, Tamil Nadu on 26th July 2015. There was no improvement, and five days after undertaking treatment at ICHH, the boy died on 1st August. Later, it was reported that the sample collected from the boy was found positive for JE.

The State Health Dept. carried out vector survey and fogging operation in the village to contain mosquito abundance and sensitized the agriculture department to promote intermittent irrigation in the rice fields.

At the request of the Health Department, VCRC team carried out an investigation at Keezh Sathamangalam pet, Puducherry on 5th August 2015 to assess the environmental and entomological risk factors for JE transmission in the area.

Environmental risk: The boy was a resident of Keezh Sathamangalam pet, near Ariyur, (under Villianur Commune Panchayat), Puducherry. The village is located at a distance of 14 Km west of Puducherry town. On enquiry of the family members, it was learnt that the boy and his family members did not visit any of the known JE endemic areas in the past one month i.e., prior to the onset of fever.

There are 302 holdings in the village with a population of 1200. About 50 cattle were present in the village and the man to cattle ratio was 1:0.04. The village is surrounded by vast areas of paddy fields, and the rice plants were about one month old during our visit.

No pig was seen in the village during the investigation. However, pigs are reared in Gorkadu village, situated at a distance of 2.5 Km. On enquiry, it was learnt that there is a duck farm close to the village and the wading birds move around the neighbouring villages.

Entomological risk: Mosquitoes resting in human dwellings and cattle sheds were collected using mechanical aspirators during dusk. Simultaneously, mosquitoes resting outdoors were also collected using Backpack aspirator and sweep nets. The mosquitoes were brought to laboratory, identified, sorted according to the abdominal conditions and pooled in vials for detection of JE virus by PCR.

In total, 290 mosquitoes belonging to 11 species were collected. Of these, *Cx. tritaeniorhynchus*, the major vector of JE constituted 78% of the total mosquitoes collected. The abundance of *Cx. tritaeniorhynchus* females was 32 per man-hour in human dwellings and cattle sheds and 65 per man-hour in outdoors.

RT-PCR test results: A total of 226 *Cx. tritaeniorhynchus* mosquito samples, pooled in 13 vials were screened. All the mosquito samples tested were negative for JE virus by RT-PCR.

Conclusion: The Keezh Sathamangalampet village with extensive rice cultivation favours profuse breeding of *Cx. tritaeniorhynchus* and therefore adult population was abundant in the village during the investigation. Though not in large numbers, pigs and ducks are present in the nearby villages. Therefore, the village is receptive for transmission. The RT-PCR results have not shown any positive for JE virus in samples of vector mosquitoes. However, absence of infection in vector mosquitoes at a given point of time with limited samples will not rule out the absence of JE transmission that could have occurred earlier. It is also rare to observe infection in mosquitoes during the post episode duration. Further, the village is bordered with Cuddalore and Villupuram districts of adjacent Tamil Nadu State, where sporadic cases of JE have been reported for the last five years. Therefore, regular monitoring of incidence of cases in the area and vector surveillance is necessary. The VCRC is continuously monitoring the vector density in the area in collaboration with the State NVBDCP.

3.3 FACILITIES

3.3.1. Laboratory animal facility

The laboratory animal house facility of VCRC is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests, Government of India. The facility has breeding colonies of animals such as BALB/c mice (Mus musculus), mongolian gerbils (Meriones unguiculatus), and multimammate rats (Mastomys coucha). These animal species are being used for various research projects after getting approval of the Institutional Animal Ethics Committee (IAEC). Presently, there are four ongoing projects in which the animals are used for experiments. Another four projects using laboratory animals were completed during the reporting period. Health status of the animals is being monitored by a visiting veterinary doctor.

3.3.2. *Brugia malayi* (sub-periodic) filarial parasite colony

Brugia malayi sub-periodic strain filarial parasite is being maintained in two animal models such as mongolian gerbil (*Meriones unguiculatus*) and multimammate rat (*Mastomys coucha)*. Larval stages of the parasite (L1 to L3) are developed in *Aedes aegypti* (Liverpool strain) mosquitoes by feeding them on microfilaraemic blood collected from infected animals. The infective larvae (L3) harvested from these mosquitoes are inoculated to the animal models for development to adult worms. As on date, there are 20 numbers of multimammate rats and 5 mongolian gerbils positive for the filarial parasite infection. Adults and microfilariae collected from these animals are used for various projects on antifilarial drug development.

3.3.3. 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 3.3. Forty nine lymphoedema patients visited first time which includes patients referred from territory care hospitals like JIPMER and IGGH Puducherry. Fourteen ADLA patients received complete treatment and these patients were followed up till the disappearance of clinical signs. Most of the ADLA patients recovered by first line of antibiotics and penicillin continue to be the most prescribed antibiotics. Less than 10% of the ADLA episodes required second line of antibiotics for complete recovery. Other than the cure for ADLA episodes, patients attending filariasis clinic in VCRC are satisfied for getting

Table 3.3 Number of patients who availed the clinical services during Jan–Oct 2015

Clinical Diagnosis	Number of Patients		
	First Visit	Repeat Visits	Total Visits
LE Grade-I	3	5	8
LE Grade-II	26	154	180
LE Grade-III	14	236	250
LE Grade-IV	6	113	119
Others*	36	11	47
Total	85	519	604
ADLA#	14	36	50

*Others include non-filarial skin infections, trauma etc...
#Acute dermato-lymhangio-adenitis

specific attention for lymphoedema and since it is an exclusive clinic for filariasis the stigma is completely eliminated. Post graduate physiotherapy students of MTPG & RIHS, Puducherry provided physiotherapy services to all lymphatic filariasis patients. During this reporting period, we referred four cases for higher institutions: One case of multiple lymphadenitis referred to CMC Vellore; One case of grade IV lE was referred to GH for debulking; One case adolescent boy was referred to the department of Vascular surgery, MMC Chennai and one case of gross anaemia was referred to JIPMER.

3.3.4. 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 mosquitoes were supplied to various divisions of the Centre for carrying out basic studies on biology and susceptibility to insecticides and biocides. In addition, mosquito specimens were also supplied to Govt. High Schools for conducting exhibition to create awareness about vector borne diseases, mosquito life stages and their control.

Mosquitoes (Diptera: Culicidae)

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

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

- *Gambusia affinis*
- *Poecilia reticulata*

Supplies from Rearing & Colonization Division to other laboratories of VCRC and outside institution are given in Table 3.4.

Table 3.4 Supplies from Rearing & Colonization Division to different laboratories during Jan–Sep 2015

Species	Internal (within VCRC)					External*	Total
	Vector Biology & Control	Microbiology, Immunology & Bioinformatics	Human Resource Development	Chemistry	Vector Ecology & Surveillance	Govt. High Schools, Puducherry	
Culex quinquefasciatus							
Immature stages	–	136700	43650	300	–	–	**180650**
Adults	600	500	3050	–	100	–	**4250**
Anopheles stephensi							
Immature stages	3150	23475	12600	–	–	–	**39225**
Adults	19075	200	3300	–	100	–	**22675**
Aedes aegypti							
Immature stages	1700	15650	13800	100	–	–	**31750**
Adults	600	–	7040	2100	100	–	**10340**
Toxorhynchites splendens							
Immature stages	–	–	–	–	–	100	**100**
Adults	–	–	–	–	–	50	**50**

*Supplied for Exhibition purpose


MOLECULAR CELL BIOLOGY
Statistics
Probability and Statistical Inference
INTERMEDIATE STATISTICS
CALCULUS
OPERATIONS MANAGEMENT
OPERATIONS RESEARCH
Principles of Operations Research
statistical tables
SYSTEMS ANALYSIS AND SIMULATION IN ECOLOGY
MANAGEMENT
WORKSHOP STATISTICS
INTRODUCTION TO BIOMETRY
SPATIAL ECOLOGY
Handbook of Spatial Statistics
STATISTICAL ADVANCES IN THE BIOMEDICAL SCIENCES
RESEARCH AND DEVELOPMENT STATISTICS
ECONOMIC TABLES

Unit 4
Publications

1. Rajavel AR, Pradeep Kumar N, Natarajan R, Vanamail P, Rathinakumar A and Jambulingam P. Morphological and molecular characterization of the ecological, biological and behavioural variants of the JE vector *Culextritaeniorhynchus:* An assessment of its taxonomic status. *Journal of Vector Borne Diseases,* 2015, 52:40–51.
2. Srinivasan R, Jambulingam P, Pradeep Kumar N, Selvakumar M and Edwin B. Temporal distribution and behaviour of sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus of the Kani Tribe settlements in the Western Ghats, India. *Acta Tropica,* 2015, 148:147–155.
3. Pradeep Kumar N, Srinivasan R, Anish TS, Nandakumar G and Jambulingam P, Cutaneous leishmaniasis caused by *Leishmania donovani* in the tribal population of the Agasthyamala Biosphere Reserve forest, Western Ghats, Kerala, India. *Journal of Medical Microbiology,* 2015, 64:157–163.
4. Mittu Thomas and Pradeep Kumar N. Micro-evolutionary trends of dengue virus-3, in Kerala state, India. *Indian Journal of Experimental Biology,* 2015, 53:236–240.
5. Bhuvaneswari S, Manonmani AM and Geetha I. Cost effective medium for the production of mosquito pupicidal lipopeptide from *Bacillus subtilis* subsp. s*ubtilis* (VCRC B471). *Journal of Vector Borne Diseases,* 2015, 52:58–62.
6. Prabakaran G, Hoti SL, Surya Prakash Rao and Satish V. Di-rhamnolipid is a mosquito pupicidal metabolite from *Pseudomonas fluorescens* (VCRC B426). *Acta Tropica,* 2015, 148:24–31.
7. Shriram AN, Krishnamoorthy K and Vijayachari P. Diurnally subperiodic filariasis among the Nicobarese of Nicobar district - epidemiology, vector dynamics & prospects of elimination. *Indian Journal of Medical Research,* 2015, 141:598–607.
8. Jeelani S, Sabesan S and Subramanian S. Community knowledge, awareness and preventive practices regarding dengue fever in Puducherry. *Public Health,* 2015, 129(6):790–796.
9. Poopathi S, De Britto RLJ, Thirugnana sambantham K, Ragul K, Mani C and Balagangadharan K. An investigation on the diversity of mosquitocidal bacteria and its relationship with incidence of vector borne diseases. *Tropical Biomedicine,* 2015, 32(1):84–97.
10. Srinivasan R and Subramanian S. Sandfly species diversity in association with human activities in the Kani tribe settlements of the Western Ghats, Thiruvananthapuram, Kerala, India. *Mem Ins Oswaldo Cruz, Rio de Janeiro.* 2015, 110(4):174–180.
11. Chauhan N, Sharma R and Hoti SL. Identification and biochemical characterization of macrophage migration inhibitory factor-2 (MIF-2) homologue of human lymphatic filarial parasite, *Wuchereria bancrofti. Acta Tropica,* 2015, 142:71–78.
12. Mary KA, Paily KP, Hoti SL and Balaraman K. Binding sites of mosquitocidal toxins of *Pseudomonas fluorescens* and *Bacillus subtilis* on pupae and larvae of *Culex quinquefasciatus. J Immunoassay Immunochem,* 2015, 36(1):54–62.
13. Chandhiran K and Paily KP. Natural parasitism of *Romanomermis iyengari* (Welch) (Nematoda: Mermithidae) on various species of mosquitoes breeding in rice fields in Puducherry, India. *Biol Cont,* 2015, 83:1–6.
14. Nanda Kumar Y, Jeyakodi G, Thulasibabu R, Gunasekaran K and Jambulingam P. Screening and Identification of Inhibitors Against Glutathione Synthetase, a Potential Drug Target of *Plasmodium falciparum. Combinatorial Chemistry & High Throughput Screening,* 2015, 18:492–504.
15. Nanda Kumar Y, Jeyakodi G, Gunasekaran K and Jambulingam P. Computational screening and characterization of putative vaccine candidates of *Plasmodium vivax. Journal of Biomolecular Structure and Dynamics*, 2015, 29:1–15.
16. De Britto RLJ, Yuvaraj J, Kamaraj P, Poopathi S and Vijayalakshmi G. Risk factors for chronic intertrigo of lymphoedema leg in southern India: A case-control study. *The International Journal of Lower Extremity Wounds,* 2015, DOI:10.1177/1534734615604289.
17. De Britto RLJ, Vanamail P, Sankari T, Vijayalakshmi G, Das LK and Pani SP. Enhanced efficacy of sequential administration of Albendazole for the clearance of *Wuchereria bancrofti* infection: Double blind RCT. *Tropical Biomedicine*, 2015, 32(2):198–209.
18. Aarumugam P, Krishnamoorthy N and Gunasekaran K. Deltamethrin induced functional mortality of *Anopheles stephensi*, the urban malaria vector, in relation to resistance development. *Journal of Vector Borne Diseases*, 2015, 52(3):261–264.
19. Anuse SS, Sahu SS, Subramanian S and Gunasekaran K. Usage pattern, physical integrity & insecticidal efficacy of long lasting insecticidal nets in Odisha State, India. *Indian Journal of Medical Research*, 2015, 142:71–78.

20. Mani C, Thirugnanasambantham K, Sundara pandian SM and Poopathi S. Identification and characterization of a novel marine *Bacillus cereus* VCRC-B540 for mosquito control. *Biocontrol*, 2015, 60(1):71–79.
21. Poopathi S, De Britto RLJ, Praba CL, Mani C and Praveen M. Synthesis of silver nanoparticles from *Azadirachta indica*-a most effective method for mosquito control. *Environmental Science and Pollution Research*, 2015, 22(4):2956–2963.
22. Saratha R and Nisha M. Development of a mosquito attractant blend of small molecules against host-seeking *Aedes aegypti*. *Parasitology Research*, 2015, DOI 10.1007/s00436-015-4886-0.
23. Tamilselvan S, Jambulingam P, Manoharan V, Shanmugasundaram R, Vivekanandan G and Manonmani AM. Fly ash based *Bacillus thuringiensis* var. *israelensis* formulation for use against *Culex quinquefasciatus*, the vector of filariasis in natural ecosystems. *Journal of Vector Borne Diseases*, 2015, 52(3):193–200.
24. Sahu SS, Gunasekaran K and Jambulingam P. Field performance of malaria rapid diagnostic test for the detection of *Plasmodium falciparum* infection in Odisha State, India. *Indian Journal of Medical Research*, 2015, 142:52–58.
25. Sahu SS, Gunasekaran K, Vijayakumar T and Jambulingam P. Triple insecticide resistance in *Anopheles culcifacies*; a practical impediment for malaria control in Odisha State, India. *Indian Journal of Medical Research*, 2015, 142:59–63.

Accepted

26. Raju KHK, Sabesan S, Rajavel AR, Subramanian S, Natarajan R, Thenmozhi V, Tyagi BK and Jambulingam P. A preliminary study to forecast *Japanese encephalitis* vector abundance in paddy growing area, with the aid of radar satellite images. *Vector-Borne and Zoonotic Diseases*, 2015 (in press).

Unit 5

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

Date	Particulars	Scientist
2–17 Jan	DBT sponsored training course on '*Fermentation and Bioseparation Methods*' organized by Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry	Dr. I. Geetha
7 Jan	Tribal Health Research Forum - Stakeholders meeting, ICMR, New Delhi	Dr. P. Jambulingam Dr. K. Gunasekaran
19 Jan	Interactive meeting on Thrust areas of research by ICMR Tribal Health Research Unit, VCRC, Puducherry	Dr. K. Gunasekaran Dr. C. Sadanandane
31 Jan	Meeting of the three ICMR institutes on vector mapping held at RMRC, Bhubaneswar	Dr. P. Jambulingam Dr. K. Gunasekaran
2–13 Feb	WHO consult to assist Republic of Maldives to prepare dossier for LF elimination	Dr. K. Krishnamoorthy
9 Feb	District Level Inter Sectoral Co-ordination Committee Meeting on control of vector-borne diseases organized by District Collector, Kottayam	Dr. N. Pradeep Kumar
11 Mar	Task force meeting on insecticide resistance monitoring at ICMR Hqrs.	Dr. P. Jambulingam
11 Mar	Participated and presented 2 indigenous technologies at the exhibition on 'Innovation in Medical Science & Technology' held at Rashtrapati Bhavan, New Delhi	Dr. I. Geetha Dr. A.M. Manonmani
18 Mar	Stakeholders meeting to launch the DEC fortified salt in Nancowry islands of the collaborative project "Effectiveness and operational feasibility of mass DEC fortified salt as a supplementary intervention to MDA towards elimination of the lone foci of diurnally subperiodic *W. bancrofti* in Andaman & Nicobar Islands" in RMRC, Port Blair	Dr. P. Jambulingam
24 Mar	First meeting of the Regional Task Force on Diseases Targeted for Elimination before 2020 at WHO SEARO, New Delhi	Dr. P. Jambulingam
26–28 Mar	Training on "Kyasanur Forest Disease (KFD)", at Shimoga, Karnataka- Participated as a resource person, delivered a lecture on KFD and imparted training on collection and identification of Ticks to Entomologists and District Surveillance Officers, Govt. of Karnataka	Dr. C. Sadanandane
28 Mar	CME Programme on 'Blood stream infections –An update, organized by International Medical Sciences Academy - Puducherry Chapter and Mahatma Gandhi Medical College and Research Institute, Puducherry	Dr. I. Geetha
28 Mar	Seminar on "Biodiversity and Conservation initiatives in Koraput region, Odisha" conducted at Central University, Koraput	Dr. S.S. Sahu
29 Mar	Delivered a lecture on 'Biodiversity and Persistence of Malaria in Koraput district' at Central University, Koraput	Dr. S.S. Sahu
2 Apr	Seminar on Trends and techniques in Zoology, delivered a lecture & keynote address in Kanchi Mamunivar Centre for PG Studies, Puducherry	Dr. P. Jambulingam
15 Apr	Expert Group meeting on Insecticides held at NVBDCP, Delhi	Dr. P. Jambulingam Dr. K. Gunasekaran
15 Apr	District level malaria review meeting at CDMO chamber, Koraput under the chairmanship of the Joint Director, Malaria Bhubaneswar	Dr. S.S. Sahu
16–17 Apr	Training Workshop of Liaison Officers for SCs/STs for ICMR, conducted at the Institute of Secretariat Training and Management, Department of Personnel and Training, New Delhi	Dr. K.P. Paily
20 Apr	Recent Advances in Laboratory Medicine organized by Mother Theresa Postgraduate & Research Institute of Health Sciences, Puducherry	Dr. I. Geetha Mr. S. Muthukumaravel Mr. A. Mathivanan
20–26 Apr	Training workshops on MDA for district level officials in six localities covering 24 districts in Bihar	Dr. K. Krishnamoorthy
20 May	Meeting with the State program officers to tackle the outbreak situation of KFD & visceral Leishmaniasis occurred in Wyanad & Mallapuram districts of Kerala	Dr. N. Pradeep Kumar
5 Jun	3rd National conference on LF elimination 2015 -Mission possible in CRME, Madurai	Dr. P. Jambulingam
23–26 Jun	Regional workshop on integrated monitoring and epidemiological assessment of MDA in GPELF with special reference to use of new FTS and integrated assessment of STH infections, a Regional TAS training workshop jointly organized by VCRC & WHO/SEARO	Dr. K. Krishnamoorthy Dr. K. Gunasekaran Dr. K.P. Paily Dr. S. Subramanian Dr. V. Vasuki Mrs. A. Srividya
29–30 Jun	Meeting of Experts to discuss the road-map for research on Scrub typhus, suspected to be a major etiological agent for AES/JE in Gorakhpur region	Dr. P. Jambulingam
14–17 Jul	National Training Workshop on Integrated Monitoring and Epidemiological Assessment of MDA in GPELF with special reference to use of new FTS and Integrated Assessment of STH Infections, organized by VCRC and sponsored by the WHO Country Office, Delhi held at Puducherry	Dr. K. Krishnamoorthy Dr. K. Gunasekaran Dr. K.P. Paily Dr. S. Subramanian Dr. V. Vasuki Mrs. A. Srividya

Date	Particulars	Scientist
23–24 Jul	Workshop on "Access and retrieval techniques of full text from ICMR e-consortia and J Gate Plus" organized by the Regional Medical Research Centre, Port Blair	Dr. R. Srinivasan
28 Jul	Institutional Biosafety Committee meeting of Krishi Vigyan Kendra, Puducherry	Dr. K.P. Paily
28–31 Jul	Plasmodium vivax Global meeting & Informal Consultation on Adaptation of Malaria Global Technical Strategy in WHO South-East Asia Region, New Delhi	Dr. P. Jambulingam
31 Jul	Expert Review Group Meeting for evaluation ofpublic health pesticides, ICMR, Delhi	Dr. K. Gunasekaran
18 Aug	Meeting of the "Expert Group" to discuss proposals on detecting the role of scrub typhus in the etiology of AES/JE in Gorakhpur, UP, held at ICMR Hqrs. New Delhi	Dr. K.P. Paily Dr. C. Sadanandane
19 Aug	Meeting of the Expert group on developing course curriculum for training courses on specific vectors, at ICMR Hqrs. New Delhi	Dr. C. Sadanandane
19 Aug	Meeting to finalize the validation protocol and handing over the insecticide impregnated papers to external evaluators held at ICMR HQ, New Delhi	Dr. Nisha Mathew
24 Aug	Meeting at Health Secretariat, Bhubaneswar on "participation of TATA Trust on malaria control in ten southern districts of Odisha" under the chairmanship of Health Secretary, Govt of Odisha organised jointly by NVBDCP, New Delhi and Health Department, Govt. of Odisha	Dr. S.S. Sahu
2–4 Sep	NVBDCP-WHO Training Workshop on Transmission Assessment Survey in Thiruvananthapuram, Kerala	Dr. K. Krishnamoorthy
4 Sep	Directors' Meeting at NIE Chennai	Dr. P. Jambulingam
8–10 Sep	NVBDCP-WHO Training Workshop on Transmission Assessment Survey, Patna, Bihar	Dr. K. Krishnamoorthy
16 Sep	Institutional Animal Ethics Committee meeting of Pondicherry University	Dr. K.P. Paily
21 Sep – 9 Oct	National level training workshop on "Geospatial Technology and its application" organized by KSCST, Bangalore at IISC Bangalore	Mrs. A. Srividya
28–30 Sep	Regional Training Workshop on Transmission AssessmentSurvey (TAS) under ELF programme& Integrated assessmentof STH prevalence organized by the NVBDCP and the WHOCountry Office, Delhi, Jabalpur, Madhya Pradesh	Dr. K. Gunasekaran
28–30 Sep	Meeting and symposium conducted for all preceptors of undergraduate practical experience abroad and MScGH Scholarly Project abroad program at Georgetown University, Washington DC, USA	Dr. A.M. Manonmani
2–4 Oct	NVBDCP-WHO Training Workshop on Transmission Assessment Survey, conducted at Thiruvananthapuram, Kerala	Dr. K.P. Paily
5–9 Oct	Public Training programme on IPRs conducted by Rajiv Gandhi National Institute of Intellectual Property Management, Nagpur	Dr. V. Vasuki
16 Oct	Task Force meeting convened by Hon'ble Minister for Local Administration, Govt. of Puducherry	Dr. K. Gunasekaran
16 Oct	Institutional Human Ethics Committee (IHEC) of Siddha Regional Research Institute (SRRI), Puducherry	Dr. R.L.J. De Britto
20 Oct	Expert Committee meeting on the increasing incidence of Scrub Typhus in Kerala state, at the DHS Office, Kerala	Dr. N. Pradeep Kumar
2–9 Oct	26th AICZ and International Symposium-2015, Lucknow	Dr. S. Poopathi
2 Nov	Brainstorming Workshop on Integrated Vector Management organized by NVBDCP & WHO, NCDC, Delhi	Dr. K. Gunasekaran
3 Nov	Seminar on Genomics & Proteomics of Disease Vectors and Pathogens organised by Bio-medical Informatics Centre (ICMR), at VCRC, Puducherry	Dr. Nisha Mathew Dr. K. Athisayamary Dr. A.M. Manonmani Mr. S. Muthukumaravel Mrs. T. Sankari Mr. A. Mathivanan
5–6 Nov	Public Training programme on Patent Search conducted by Rajiv Gandhi National Institute of Intellectual Property Management, Nagpur	Dr. A.M. Manonmani
20 Nov	Launch of Distribution of Double Fortified salt (DEC+Iodine) for elimination of filariasis to the community at risk , RMRC, Port Blair	Dr. P. Jambulingam
28 Nov	Chaired the oral paper session on 'Immunology & Molecular Techniques' at the 39th Annual Conference of Indian Association of Medical Microbiologists – Microcon 2015, JIPMER, Puducherry	Dr. A.M. Manonmani
10–11 Dec	Finalization of MoU between VCRC & NRSC, Hyderabad on Space Technology Applications for Vector Borne Disease Control held at NRSC, Hyderabad	Dr. P. Jambulingam


CLEAN
INDIA
CAMPAIGN
October 2, Friday 2015
Vector Control Research Centre
Indian Council of Medical Research
Puducherry
स्वच्छ भारत अभियान
VECTOR CONTROL
organi
"कर सेवा"
October 2

Unit 6
Celebrations

6.1 OFFICIAL LANGUAGE IMPLEMENTATION

राजभाषा (हिन्दी) वार्षिक रिपोर्ट – 2015

आई. सी. एम. आर (ICMR) महानिदेशक की अपील पर, केंद्र मे दो दिनों दिनांक 23/09/2015 और 24/09/2015 पर हिन्दी दिवस सफलतापूर्वक मनाया गया था । केंद्र की कर्मचारियों और छात्रों के बीच विभिन्न प्रतियोगिताओं आयोजित की गई। गायन, अच्छा हस्तलिपी, सब्द अनुवाद, भाषण और सब्द सक्ति प्रतियोगीता आयोजित की गई। डॉ. सी. जै संकर बाबु, सहायक प्रोफेसर, हिन्दी विभाग, पोण्डिचेर्री विश्वविद्यालय, पुधुच्चेरी, ईस अवसर पर वे मुख्य अतिथि थे और वे एक व्याख्यान "राजभाषा के बेहतर क्रियान्वयन के लिए सूचना प्रौद्योगिकी का उपयोग" पर दिए थे और प्रतियोगिताओं के विजेताओं को पुरस्कार वितरण किये । इसके अलावा कर्मचारियों ने नगर राजभाषा कार्यान्वयन समिति (TOLIC), जीपमार (JIPMER) द्वारा आयोजित हिन्दी पखवाड़े उत्सव के अवसर पर आयोजित विभिन्न प्रतियोगिता में भाग लिये ।

ईस वर्ष हिंदी शिक्षण योजना में कर्मचारियों प्रशिक्षण लिये हैं, और 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 (JIMPER), पुधुच्चेरी और क्षेत्रीय कार्यालय, कोचीन को दो अर्ध – वार्षिक रिपोर्ट भेजा गया था। वर्ष के दौरान (VCRC OLIC) के सदस्य सचिव जीपमार (JIMPER), पुधुच्चेरी में नगर राजभाषा कार्यान्वयन समिति के बैठकों में भाग लिये ।

6.2 INTERNATIONAL WOMEN'S DAY

International Women's Day (IWD) was celebrated on March 8, 2015 with a theme "Make It Happen". The event highlighted women's achievements, recognized challenges, and focused greater attention on women's rights and gender equality to mobilize all people to do their part. It also envisioned a world where each woman and girl can exercise her choices, such as participating in politics, getting an education, having an income, and living in societies free from violence and discrimination. The Chief Guest of this occasion Mrs. P.D. Mageswari, Physiotherapist, SangeethShantham Foundation for Mentally Retarded Children, delivered a talk on "Stress free life and good health", a topic relevant to the occasion. As a part of welfare measure to our women employees of VCRC, health screening (pap smear test and mammogram) was carried out at JIPMER to detect early cancer signs. The celebration gave a message to galvanize action to address the gaps that still remain in making gender equality a reality.

6.3 INTERNATIONAL DAY FOR YOGA

A two day programme was organized in commemoration with "Celebration of International Day for Yoga" at Vector Control Research Centre, Puducherry. To sensitize our staff, post graduate students, Ph.D. scholars and family members of staff participated and yoga Day Special lecture was organized. Mr. S. Murugaiyan, Managing Trustee, World Community Service Centre (WCSC), Temple of Consciousness, Lawspet, Pondicherry, founded by Shri Vethathiri Maharishi delivered a lecture on "Science of Yoga and Meditation system" on Friday, the 19th June 2015 at 10.00 am at Dr. T. Ramachandra Rao Auditorium. The basic principles and how yoga connects the mind and body in an individual & brings harmony was discussed. Its role in stress management, disease management and disease prevention were also addressed. The lecture was followed by practical yoga class. On International Day of Yoga, the 21st June 2015 at 10.00 am, a lecture on Kaya kalpa Yoga was delivered by Mr. S. Murugaiyan. After the lecture, the different steps of Kaya Kalpa yoga were demonstrated and practical sessions were conducted.

6.4 NATIONAL SCIENCE DAY

National Science Day was celebrated on 30th March 2015 in T.R. Rao Auditorium, VCRC. Dr. Vidyaa Ramkumar from M.S. Swaminathan Research Foundation, Puducherry delivered a talk on 'Biovillages of MSSRF – A case in science in nation building'. 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.

6.5 SWACHH BHARAT

Clearing of unhygienic spots and locations: Bushes around the open drainage, Garbage collected from electrical department, Trash behind the temporary filariasis clinic settings, dusts and leaves collected on the open terrace of the main building, dumping of waste behind the staff quarters, Wastes dumped in men's hostel room in ground floor, soiling of areas on the sunshades, birds soiling on the vehicle carriages had been identified and cleaned. Two spots dumped with the materials for auction were cleared through auction. Old electrical fittings and furniture placed under the staircase have been cleared after undertaking repair work. Refrigerator provided in the canteen have been completely deloaded and cleaned.

Organization of competition for school children: We have conducted an essay competition to class 6–9 School children in Govt. Higher secondary school Indira nagar, Puducherry, Three students have been selected for prize distribution for the essay competition.

Identification of demonstration village under Swachh Bharat: Two teams visited various villages around Puducherry and observed that Swach Bharat is also implemented by local Govt. However, one gypsy colony provided with the Govt. housing has been identified as a potential area for adaptation.

Organization of voluntary 'KarSewa: We had 'KarSewa' in around VCRC office and Quarters by the 2nd week of October 2015.

Weeding out old files: A total of 1271 case booklets of filariasis patients that are more than 25 years old and brittle, Old case sheets of patients not visiting more than 10 years have been segregated were weeded out. 338 files have been identified and weeded out.

Auctioning of old equipments / furniture / other item: Condemned items such as minor lab equipment, old furniture, UPS and Stabilizer. Office equipment - 11, Electrical equipment - 5, Lab equipment - 16, Scraps items – 1 lot, Furniture – 5 were auctioned following GFR.

Additional maintenance work carried out: Special efforts were taken to maintain the garden in order to participate in the Farm fest 2016 flower show conducted by Government of Puducherry and won 2nd prize. Apart from the routine cleaning extra efforts were taken to remove the stains in the corridor from the first and second floors. Apart from the routine cleaning, special efforts were made to clean all the fan fittings in the rooms and switch rooms in the ground floor, First floor and second floor staff quarters and the ground floor corridor.

Unit 7
Institutional Committees

7.1 EXTERNAL COMMITTEES

7.1.1. Members of 37th SAC meeting of VCRC

Prof. A.P. Dash
Chairman
Vice Chancellor
Central University of Tamil Nadu,
Thiruvarur – 610 101

Dr. A.C. Dhariwal
Member
Director
National Vector Borne Disease Control Programme,
22, Shamnath Marg,
New Delhi – 110 054

Dr. D.A. Gadkari
Member
Former Director, NIV
Shilpayatan Apartment,
2/13, Erandwane,
Pune – 411 004

Dr. P.L. Joshi
Member
Former Director, NVBDCP
Faculty (Part time)
National Institute of Health & FW,
580, HIG, Metroview Apartments,
Sector 13, Pocket B, Dwarka,
New Delhi – 110 078

Dr. M.P. Kaushik
Member
Former Director, DRDE
A 25, Govindpuri,
Mandir Marg, Thatipur,
Gwalior – 474 011

Prof. (Dr) R.C. Mahajan
Member
S.N. Bose INSA Research Professor & Emeritus Professor
House No.276, Sector 6,
Panchkula,
Haryana – 134 109

Prof. K. Ramachandran
Member
Former Prof. of Biostatistics, AIIMS
Flat 122, DSR, "Wood Winds"(Near WIPRO),
Sarjapur Road, Doddakanahalli,
Bengaluru – 560 035

Dr. Sarala K. Subbarao
Member
Consultant, Vector Science Forum
(Former Director, NIMR)
Indian Council of Medical Research,
Ansari Nagar,
New Delhi – 110 029

Dr. R.S. Sharma
Member
Additional Director & Head (Retired)
Centre for Medical Entomology & Vector Management,
National Centre for Disease Control,
22 Sham Nath Marg,
New Delhi – 110054

Dr. R.P. Swaminathan
Member
Sr. Prof. & Head
Department of Medicine, JIPMER,
Puducherry – 605 006

Dr. Rashmi Arora
ICMR Representative
Scientist 'G' & Head, ECD Division
Indian Council of Medical Research,
Ansari Nagar,
New Delhi – 110 029

7.1.2. 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,
44, Lawspet Main Road, Pudupet,
Puducherry – 605 008

Dr. S. Gunasekaran
Member
Dean (Retd.) of Humanities Studies
Pondicherry University, Block C-6,
Flat No.79, Kendriya Vihar - Phase 2,
Paruthipattu, Avadi,
Chennai – 600 071

Dr. Shanthi Ananthakrishnan
Member
2-A Vairam Enclave, Iyyanar Koil Street,
Ellapillaichavadi,
Puducherry – 605 005

Dr. L. Solomon Raja
Member
Associate Professor (Retd.)
Dr. B.R. Ambedkar Govt. Law College,
Mathur Road, Kalapet,
Puducherry – 605 014

Dr. M. Kalyanasundaram
Member
Scientist 'G' (Retd.)
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

Dr. G. Sivagnanam
Member
Prof. & Head, Dept. of Pharmacology
Indira Gandhi Govt. Medical College &
Research Institute, Kadirkamam,
Puducherry – 605 009

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

7.1.3. Institutional Animal Ethics Committee (IAEC) Members

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

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

Dr. B. Kumaran
Scientist from outside the Institute
Principal
Indira Gandhi College of Arts & Science,
Kathirkamam, Puducherry – 605 009

Mr. L.V. Prasad Reddy
Non scientific socially aware member
D. No: 2. First Floor,
Vignesh Apartments, Vasan Nagar,
Puducherry – 605 005

Dr. S. Sabesan
Biological scientist
Consultant, Scientist 'G' (Retd.)
Vector Control Research Centre,
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

7.1.4. 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
Indira Nagar,
Puducherry – 605 006

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

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

Mr. S. Balasubramanian
Member Secretary
Section Officer
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

7.1.5. Building Committee

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

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

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

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

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

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

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

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

Shri. S. Balasubramanian
Member Secretary
Section Officer
Vector Control Research Centre,
Indira Nagar,
Puducherry – 605 006

7.2 INTRA-INSTITUTIONAL COMMITTEES

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*

Recruitment Cell

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. A.R. Rajavel	*Member*
Mrs. Vasumathi Nagarajan	*Member Secretary*

General Maintenance Committee

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. A.R. Rajavel	*Member*
Dr. V. Vasuki	*Member*
Shri. S. Balasubramanian	*Member Secretary*

Condemnation Committee

Dr. R. Srinivasan	*Chairman*
Dr. C. Sadanandane	*Member*
Dr. B. Nandha	*Member*
Accounts Officer	*Member*
Section Officer (Purchase & Maintenance)	*Member Secretary*

Environmental Safety Committee / Biosafety Committee

Dr. A.M. Manonmani	*Chairman*
Dr. K.P. Paily	*Member*
Dr. R.L.J. De Britto	*Member*
Dr. V. Vasuki	*Member*
Dr. C. Sadanandane	*Member*
Shri. S. Balasubramanian	*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*
Shri. S. Balasubramanian	*Member Secretary*

Equipment Maintenance Committee

Dr. R.L.J. De Britto	*Chairman*
Dr. S. Subramanian	*Member*
Dr. Nisha Mathew	*Member*
Dr. V. Vasuki	*Member*
Shri. S. Balasubramanian	*Member Secretary*

Official Language Implementation Committee

Dr. B. Nandha	*Chairman*
Mr. B. Kumareson	*Member*
Mr. P.M. Azad	*Member*
Mrs. N. Caliany	*Member*
Mr. Y. Srinivasa 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*
Mr. S. Kandasamy	*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. Gunasekaran	*Chairman*
Dr. A.M. Manonmani	*Member*
Dr. K.P. Paily	*Member*
Dr. A.R. Rajavel	*Member*
Dr. V. Vasuki	*Member*
Dr. R. Srinivasan	*Member*
Dr. C. Sadanandane	*Member*
Accounts Officer	*Member*
Administrative Officer in-Charge	*Member Secretary*

Unit 8
Staff Position

Director

Dr. P. Jambulingam

Scientific

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*
Mrs. A. Srividya	*Scientist-D*
Dr. C. Sadanandane	*Scientist-C*
Dr. (Mrs.) B. Nandha	*Scientist-B*

Administration & Accounts

Mrs. Vasumathi Nagarajan	*Accounts Officer*
Mr. K. Sundararajan*	*Section Officer*
Mr. S. Balasubramanain	*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 Officer-A*
Mrs. Abidha	*Technical Officer-A*
Mr. T. Vijayakumar	*Technical Officer-A*
Dr. R. Natarajan	*Technical Officer-A*
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. S. Kandasamy	*Technical Assistant*
Mr. K. Vivekanandan	*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*
Mr. Sana Prasad Rao	*Technical Assistant*
Mr. S. Agatheswaran	*Technical Assistant*
Mrs. G. Vijayalakshmi	*Staff Nurse*

Dr. S. Sabesan *Consultant*	*Scientist-G (Retd.)*

*Retired from service on superannuation
**Re-employed after retirement (upto Sept. 2015)