ICMR - VECTOR CONTROL RESEARCH CENTRE

PUDUCHERRY

Annual Report 2016

WHO Collaborating Centre for Research and Training in Lymphatic Filariasis and Integrated Vector Management

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PREFACE

The year 2016 was yet another eventful period for Vector Control Research Centre. I am pleased to present the major activities and achievements of the Centre during this year.

Elimination of Lymphatic Filariasis (ELF) continued to be a priority in our action plan. A community based trial was undertaken in Yadgir, Karnataka to assess safety, acceptability and efficacy of adding a third drug, Ivermectin, to the existing regime of two drugs (DEC + Albendazole). The results of the study shows that it is safe and the alternate regimen is likely to be considered, once the efficacy results are available, for adopting globally and nationally to accelerate LF elimination. Further, a two stage cluster design based vector sampling strategy developed earlier for monitoring vector infection ('xeno-monitoring') was validated at a district (evaluation unit) level. The strategy is being recommended for post-MDA surveillance under the global LF elimination programme.

The VCRC, as a collaborating Centre of World Health Organization (WHO), nominated the Scientists for Good Laboratory Practice (GLP) Workshop organized at Penang, Malaysia during the mid 2016. With GLP compliance, it is expected to develop and implement research quality management system and also aims to obtain GLP accreditation for laboratories of the Centre.

Since the conventional vector control measures for dengue has become a challenge, a novel approach was initiated to develop *Wolbachia*-based vector control strategy under laboratory condition. In this regard, Indian Council of Medical Research (ICMR) - VCRC signed a Memorandum of Understanding (MoU) with Monash University, Australia to get the relevant technology transferred at the Centre.

The bio-pesticide formulation developed at VCRC using an indigenously isolated *Bacillus thuringiensis* var. *israelensis* (VCRC B17) strain has been licensed to two more Firms this year, now totaling to 13 Firms.

The academic programme viz., M.Sc. Public Health Entomology and Ph.D., courses affiliated to Pondicherry University continued. A 'Placement Cell' has been created at the Centre, and recruitment of students initiated with M/s. Reckitt Benckiser Pvt. Ltd., Gurgaon, besides referring them to Public Health Departments for various positions.

Training on Integrated Vector Management was imparted to the entomologists, nominated by NVBDCP and SEARO countries with WHO Fellowships. Short term research projects were encouraged for students from Georgetown University, Washington D.C., and St. Olaf College, Minnesota, USA, under the foreign University linkage programme.

The fund allocation for research and other important activities of the Centre was adequate to meet its commitment 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 Task Force of ICMR (VBDSF & THRF), Department of Science and Technology (Govt. of India), WHO-SEARO, NVBDCP, New Delhi, Melinda Gates Foundation sponsored various research and training programmes. Washington University, St. Louis, USA has extended technical support in the triple drug trial for clinical monitoring, good clinical practice and real time electronic data capturing system.

Scientific Advisory Committee and other Institutional Committees of the Centre continued to provide necessary inputs for strengthening the research and other supportive activities. The Directorate of NVBDCP, New Delhi, Dept. of Health & Family Welfare, Govt. of Puducherry, Tamil Nadu, Kerala, Odisha and Karnataka have given full support and cooperation in the collaborative field research activities.

I am very much grateful to the scientists and staff of our Centre for their exceptional contribution in achieving the desired objectives of our Centre.

Dr. P. Jambulingam
Director

प्रस्तावना

वर्ष 2016 अनेक महत्वपूर्ण कार्यक्रमों, परियोजनाओं आदि से भरा रहा। इस केंद्र की मुख्य उपलब्धियों और गतिविधियों को प्रस्तुत करते हुए मुझे अपार हर्ष है ।

हमारी प्रमुख प्राथमिकता वैश्विक और राष्ट्रीय कार्यक्रम के तहत लिंफेटिक फ़ाइलेरियासिस उन्मूलन रही। कर्नाटक के यादगीर में पहले प्रयुक्त दो दवाओं (DEC + ALBENDAZOLE) के स्थान पर इवेर्मेकीटीन (Ivermectin) नामक दवा के तमाम प्रभावों जैसे सुरक्षा, स्वीकार्यता, प्रभाव के लिए एक सामुदायिक परीक्षण किया गया। अध्ययन से पता चलता है कि यह दवा सुरक्षित तो है ही इसके प्रभावी होने के परिणाम मिलने पर राष्ट्रीय और अंतर्राष्ट्रीय स्तर पर लिंफेटिक फ़ाइलेरियासिस उन्मूलन को गति मिल सकेगी ।

इस से आगे एक जिले के स्तर पर वेक्टर संक्रमण की निगरानी (Xenomonitoring) करने के लिए द्विस्तरीय क्लस्टर डिजाइन पर आधारित वेक्टर नमूनों को एकत्र करने की कार्ययोजना को कार्यरूप दिया गया इस कार्ययोजना की सिफ़ारिश ग्लोबल लिंफेटिक फ़ाइलेरियासिस उन्मूलन कार्यक्रम के तहत पोस्ट एम डी ए सर्वेलेंस के लिए की जा रही है ।

रोग वाहक अनुसंधान केंद्र (VCRC) ने वर्ष 2016 के मध्य में विश्व स्वास्थ्य संगठन के सहयोग से पेनाग, मलेशिया में आयोजित गुड लबोरेटरी प्रैक्टिस (GLP) कार्यशाला मे भाग लेने के लिए अपने वैज्ञानिकों को नामित किया। इसके अनुपालन में यह अपेक्षा रही कि हमारे केंद्र की प्रयोगशालाओं के परीक्षण GLP के मापदंड पर खरे उतरें ।

जैसे रोग वाहक अनुसंधान केंद्र (VCRC) ने डेंगू के संक्रमण को एक चुनौती के रूप में लिया है तदनुसार वोल्बाशिया पर आधारित वेक्टर कंट्रोल की नीति को विकसित करने के उद्देश्य से प्रयोगशालाओं में सार्थक प्रयास किए गए। इसके लिए आईसीएमआर- वीसीआरसी और मोनाश विश्वविद्यालय आस्ट्रेलिया के बीच संगत तकनीक प्रदान करने के लिए एक आपसी सहमति के आधार पर MOU पर हस्ताक्षर किए गए ।

जैविक कीटनाशक के सृजन को रोग वाहक अनुसंधान केंद्र (VCRC) पर विकसित किया गया है। इसके लिए Isolated *Bacillus thuringiensis var israelensis* (VCRC B 17) स्ट्रेन का प्रयोग करने हेतु इसमें दो और फ़र्मों को लाइसेन्स दिया गया है जिनकी कुल संख्या अब 13 हो गई है ।

अकादमिक कार्यक्रम जैसे एम एस सी (M.Sc.) पब्लिक हेल्थ एंटमोलाजी और पी एच डी (Ph.D.) पाठ्यक्रम जो कि पांडिचेरी विश्वविद्यालय से सम्बद्ध हैं, उन्हें जारी रखा गया है । केंद्र पर एक प्लेसमेंट सेल भी सृजित किया गया है और विभिन्न कंपनियों से स्वास्थ्य विभाग में विभिन्न पदों के प्लेसमेंट के लिए रेफ़र करने के लिए मेसर्स रेक्किट बेंकीसर ग्रुप (M/s Reckitt Benckiser Group) के साथ छात्रों की भर्ती प्रारम्भ की गई ।

NVBDCP और SEARO देशों द्वारा नामित WHO फ़ेलोशिप के साथ एंटोमोलोजिस्तों को एकीकृत वेक्टर प्रबंधन का प्रशिक्षण दिया गया । फ़ारेन यूनिवेर्सिटी लिंकेज कार्यक्रम के तहत जॉर्जटाउन यूनिवेर्सिटी वाशिंगटन डी.सी. और सेंट ओलाफ़ कालेज मिनेसोटा, यूएसए के छात्रों को अल्पअवधि के रिसर्च प्रोजेक्ट पर काम करने के लिए प्रोत्साहित किया गया ।

अनुसंधान के लिए फ़ंड उपलब्ध करवाने और अन्य क्रियाकलापों को मूर्त रूप देने के लिए सचिव, भारत सरकार, स्वास्थ्य एवं अनुसंधान विभाग और महानिदेशक आईसीएमआर एवं ईसीडी प्रभाग आईसीएमआर नई दिल्ली के वैज्ञानिकों का अमूल्य सहयोग व समर्थन मिला है । ICMR (VBDSF & THRF) और विज्ञान एवं तकनीक विभाग (भारत सरकार) WHO (SEARO), NVBDCP नई दिल्ली, मेलिंडा गेट्स फ़ाउंडेशन के टास्क फोर्स द्वारा कई अनुसंधान और प्रशिक्षण कार्यक्रमों को प्रायोजित किया गया । वॉशिंग्टन डी सी यूनिवर्सिटी, सेंट लुईस, अमेरिका ने ट्रिपल ड्रग ट्रायल में क्लीनिकल मॉनिटरिंग, गुड क्लीनिकल प्रैक्टिस और रियल टाइम एलेक्ट्रोनिकडाटा केप्चरिंग सिस्टम के लिए तकनीकी सहयोग व समर्थन दिया ।

वैज्ञानिक सलाहकर समिति, और केंद्र की अन्य संस्थागत समितियों ने अनुसंधान एवं अन्य गतिविधियों को बल देने के लिए ज़रूरी इनपुट्स देने का काम निरंतर बनाए रखा। NVBDCP निदेशालय, नई दिल्ली, स्वास्थ्य एवं परिवार कल्याण विभाग, पुदुच्चेरी, तमिलनाडु, केरल, ओड़िशा, कर्नाटक आदि प्रदेशों की सरकारों ने विभिन्न क्षेत्रों के अनुसंधान के क्रियाकलापों के लिए पूर्ण समर्थन एवं अमूल्य सहयोग प्रदान किया है । विभिन्न लक्ष्यों को प्राप्त करने की सफलता का श्रेय मैं इस केंद्र के वैज्ञानिकों और अधिकारियों /कर्मचारियों को देना चाहूँगा जिनके अथक प्रयास से यह संभव हो सका है। मैं इन सभी के प्रति अपना आभार व्यक्त करता हूँ ।

डॉ. पी. जम्बुलिंगम
निदेशक

EXECUTIVE SUMMARY

The important research activities and major achievements during 2016–2017 are given below:

Filariasis

Development of surveillance tools for Lymphatic Filariasis Elimination Programme

- A two stage cluster design based vector surveillance strategy (sampling design, gravid-trap, PCR assay) for post-MDA vector surveillance ('xenomonitoring') developed earlier was validated in one of the evaluation units (EU) in Cuddalore district Tamil Nadu. Further validation of the strategy is in progress in 3 districts with different settings (TAS failed, TAS passed once, and twice). The strategy is being recommended to support its adoption as a standardized protocol for global LF elimination programmes.
- A community based large scale efficacy, safety and acceptability study with 3-drug regimen is being carried out in comparison to the current 2-drug regimen to find out alternate strategy to accelerate LF elimination in 'hardcore' districts. The safety data show that 3-drug regimen is safe and acceptable to communities.
- The stochastic micro-simulation model, LYMFASIM, for filariasis transmission and control was used to assess the impact of 3-drug regimen (Ivermectin, DEC & Albendazole) over the currently recommended 2-drug regimen for Mass Drug Administration for elimination of LF. The model predictions showed that mass drug administration of 3 drug regimen with coverage of 80-85% could reduce the number of MDA by one or two rounds.
- A miniaturized version of Electro Chemical-biosensor developed for the detection of filarial parasite DNA in vector mosquitoes for the first time was further improvised to make it in a portable one.

Morbidity Management and Disability Prevention

- Filarial-Lymphoedema (LE) cases enduring acute dermato-lymphangio adenitis (ADLA) episodes were grouped based on the frequency of ADLA to identify patients for administering antibiotic (penicillin or doxycycline) prophylaxis. The patients attending for review at least 3 times a year are benefited in terms of reduction in ADLA episodes and LE volume reduction. Community based study carried out in 3 sets of population in Villupuram District of Tamil Nadu has shown that regular hygiene reduces ADLA episodes significantly and also reduction in LE volume.

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 in Noncowry Islands of Andaman & Nicobar Islands, has recorded coverage over 85%. Process monitoring through random and fixed households indicate the native community is using the double fortified salt for cooking and table purpose. Salt samples tested at the household level, for DEC homogeneity indicates that the levels of DEC in the salt are in the therapeutic range.

Malaria

- Prevalence of G6PD deficiency among different tribes was studied in Odisha State. Out of 1,423 people from six tribes screened so far, 67 (4.7%) were found to have G6PD deficiency. Since, *Plasmodium vivax* is prevalent in tribal areas, the study results provide evidence that has implications on the treatment regimens to be used for the treatment of *vivax* patients in the area.
- Studies on "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" showed that the room spray (IRS) coverage and the use rate of LLIN could be enhanced to >75% through reinforced IEC activities and strengthening the advance information system about implementation of intervention measures to the villages. With the level of coverage, a significant reduction was observed in vector abundance and malaria incidence in the three arms (IRS alone, LLIN alone and combination of LLIN+IRS). However, the reduction was not significantly ($p>0.05$) different between the arms, indicating that the combination had no added advantage.

Japanese encephalitis

- Implementation of indoor residual spraying in two Blocks significantly reduced risk of transmission of JE in Gorakhpur district (UP) by reducing the vector density. In addition, around 60,000 long lasting insecticidal nets (LLINs) have been distributed in another block and its impact on JE transmission is monitored.

Dengue

- In view of developing a sustainable surveillance system for *Aedes* mosquito control, a collaborative approach involving school students has been designed with the Department of Education, Puducherry. Students were trained on assessing breeding sources in and around their houses using a simple pictorial proforma in local language. A training workshop was conducted jointly with state NVBDCP for students and teachers of selected schools on consolidation of data collected on mosquito vector breeding and for online transmission to NVBDCP for appropriate action.

Scrub Typhus

- "Prevalence of scrub typhus vectors/rodent hosts and the pathogen, *Orientia tsutsugamushi* in areas reporting human cases of AES in Gorakhpur district, Uttar Pradesh" showed high abundance of mite vector species with an estimated *Leptotrombidium deliense* index of 13.8 per animal, which was well above the critical level (0.69 per animal), in the 13 villages surveyed. The study also demonstrated natural infection of *O. tsutsugamushi* in animal hosts and vector mites in the AES reporting villages of Gorakhpur district, which confirmed transmission of scrub typhus.

Kyasanur Forest Disease

- Preliminary studies on Kyasanur forest disease virus in ticks and antibodies in rodents in potential risk areas of adjoining States to Karnataka showed high abundance of Haemaphysalis vectors in the villages in forest fringes of the six districts surveyed in Western Ghats of Tamil Nadu, Karanataka and Kerala. KFDV infection was detected by NIV in the tick vector in two of the six districts. KFD risk maps created at the district level were provided to the Programme personnel for strengthening the ongoing preventive measures such as vaccination and supply of insect repellents to the high risk groups and intensive health education

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

- *Bacillus amyloliquefaciens (B483)* exhibiting anti-mosquitocidal and anti-microbial activity*:* The metabolites of an indigenously isolated *B. amyloliquefaciens* (VCRC B483) were found to exhibit mosquitocidal, anti-bacterial and keratinase activity. Methanolic extract of the metabolites were indicative of isoforms of Bacillomycin D (1031.06, 1067.65, and 1081.77), Surfactin (1044.78, 1058.87, and 1074.67) and Fengycin (1447.69, 1463.80, 1477.83, 1491.80, and 1505.85). The metabolites were also found to exhibit antifungal activity against phytopathogens, *Fusarium sp*. (VCRC F25) and *Curvularia* sp. (VCRC F26).
- *DNA fingerprint of VCRC-B17:* The process technology related to the mosquito larvicidal biopesticide, Bti (VCRC B17) has been licenced to firms and is expected to be used for mosquito vector

control under national programme. Hence, DNA fingerprint of the strain becomes useful to monitor the use of the strain in the breeding habitats. Studies have shown that *rpoB* gene can be used as molecular/ phylogenetic marker for the identification of *B. thuringiensis* subsp israelensis.

- *Development of new mosquito control agents based on anthranillic diamides targeting the insect ryanodine receptor:* A total of ten molecules belonging to a new class of anthranillic diamide insecticides were synthesized, purified and tested against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* showed LC_{50} values below 5 ppm against larvae of all the three species. Two of them showed LC_{50} values of 0.512 and 0.7 ppm against larvae of *Cx. quinquefasciatus*, 0.337 and 0.573 ppm against *An. stephensi* and 1.282 and 1.985 ppm against larvae of *Ae. aegypti,* respectively.

Technology transfer

- A process for the preparation of mosquito larvicidal formulation from *Bacillus thuringiensis* var. *israelensis* – Technology licenced to 2 firms, Advance Crop Care Pvt Ltd, Indore; and Black Water Biotech, Mumbai during the year 2016.

Development of naphthoquinone analogues as macrofilaricidal agents.

- Based on the ADME results, six compounds TR-NPQ 1, 2, 4-7 have been short listed for in-vivo macrofilaricidal activity and Thiadiazole and Quinoline Appended Thiadiazole Derivatives as Novel Lead Candidates.

Biomedical Informatics

- *VectorInfo' a web repository of medically important Indian arthropods:* Development of a Vector Informatics Database – 'VectorInfo' – a repository of data on medically Important Indian Arthropods is continued and development of a digital data entry system for Zika/Dengue vector surveillance project is in progress.
- *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

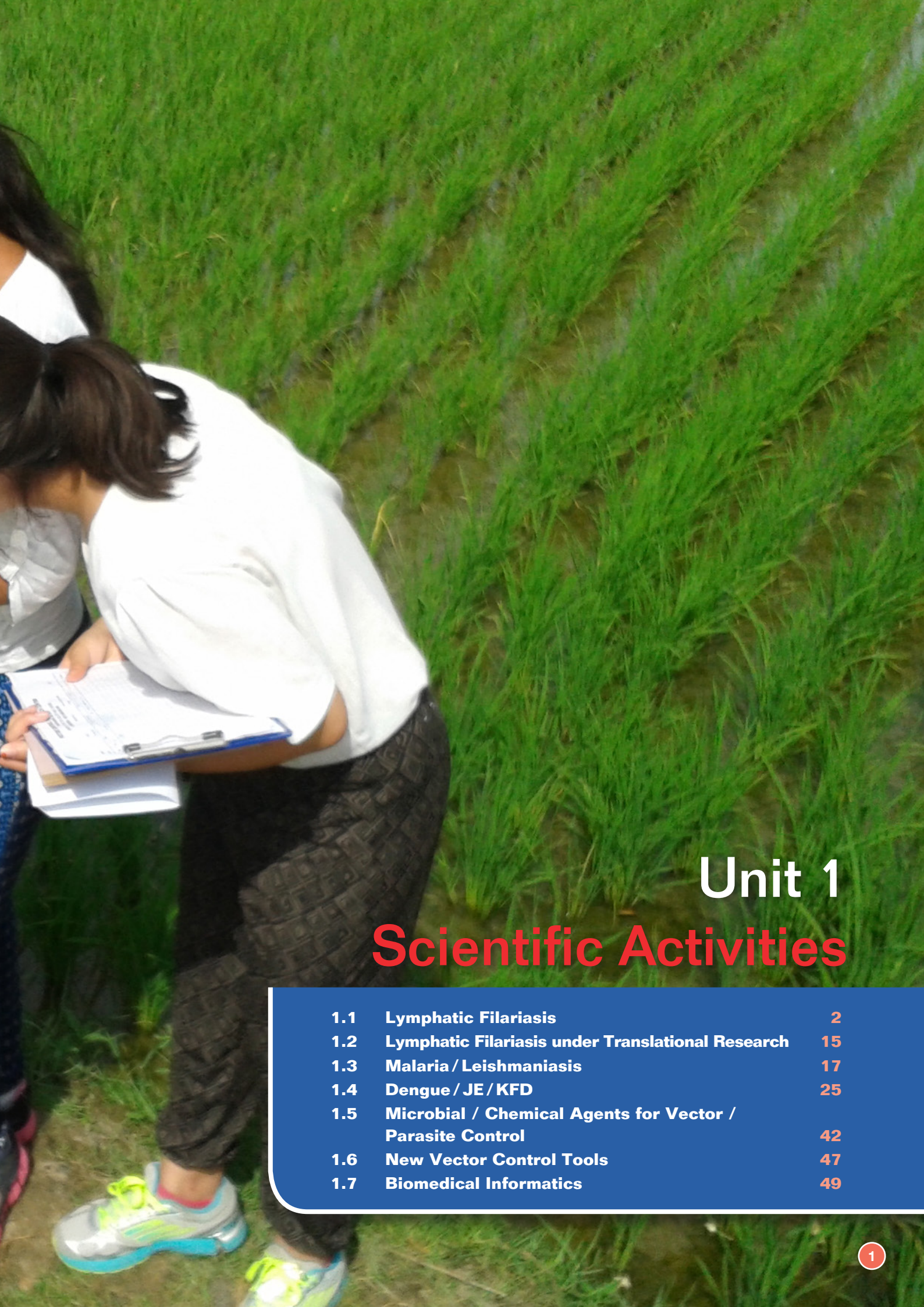
Academic

- **M.Sc. Public Health Entomology:** Twelve candidates have been admitted for the year 2016 – '18 (sixth batch) of M.Sc. Public Health Entomology course affiliated to Pondicherry University.
- **Ph.D. Programme:** Two candidates (Zoology Full time - 1 and Microbiology Part time - 1) were awarded with Ph.D. degree. Fifteen full time candidates and one part time candidate continue to pursue their doctoral programme. Two staff members have joined the Ph.D. programme as part time candidates, during the current year.

Training

- Five Scientists and Ten Technical Staff from other National Institutes were given training on vector entomology and molecular diagnostics.
- 25 Biologists / Sr. Entomologists from 10 States were provided with training on the development of Integrated Vector Management (IVM) strategy, sponsored by National Vector Borne Diseases Control Programme (NVBDCP).
- Three Health Officials from Medical Research Institute, Colombo, Sri Lanka had training on entomology with WHO Fellowships.
- Two students each from the Department of International Health, Georgetown University, Washington D.C, USA and St. Olaf College, Minnesota, USA have undertaken short term research projects.
- 17 Post-Graduate students from various Medical colleges have undergone observational training on the control of vectors and vector borne diseases.


adidas



Unit 1
Scientific Activities

1.1 LYMPHATIC FILARIASIS

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

IM 1401: Jul 2014 – Jun 2016

Das LK, De Britto RLJ, Vijayalaxmi G, Krishna Kumari A

Objectives:

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

Participants Groups: Group1 comprised of 75 patients who received morbidity management package from VCRC 5 years before and is now under state programme, Group 2 comprised of 74 patients who received morbidity management package exclusively from Tamil Nadu Government, and Group 3 comprised of 63 patients who received morbidity management package from VCRC during the study period, under supervision and follow-up.

Follow up: During this reporting period the follow up examinations were carried out in group 1 and group 2 villages. In total, 58 participants and 59 participants were examined in group 1 villages for 6 and 12 months follow up respectively. Similarly, 60 participants and 61 participants were examined in group 2 villages for 6 months and 12 months follow up respectively.

Data entry, update and cleaning: All the data recorded in both the groups were entered in excel platform and verified by one technical staff. All the missed information were noted and verified in data sheets. All the available information were entered and the data was cross checked using filter option.

Data analysis: For the year 2015, data was analysed using individual LE patients as unit. As there were 13 bilateral lymphoedema cases recruited in the study, now, the data is relooked in-terms of response taking LE leg as a unit. The clinical data is reorganized for the same. Analysis on QoL has been taken up separately and completed.

Results: In total, 60, 62 and 63 LE patients had been recruited in the study and in terms disease affected legs it was 67, 63 and 66 LE legs in group1, group 2 and group 3 respectively. Initial analysis in terms of changes in clinical parameters is given in Table 1.1. It is observed that there is a perceptible improvement in intertrigo scores.

Impact of MMDP: Comparison of Baseline HRQOL scores and HRQoL scores after one year. The Health Related Quality of Life (HRQoL) scores of the 3 groups of Lymphatic Filariasis (LF) patients who received MMDP package from three different sources were assessed using the LFQoL instrument. The base line HRQoL, and HRQoL assessed after one year (Figure 1.1) indicate that, the quality of life has come down in grade 3 and grade 4 LF patients in group 1 and 2, but not statistically significant. The QoL in LF patients in Group 3 is maintained at one year including in grade 3 and 4 LE patients. The baseline HRQoL scores and the scores after one year were almost similar. However, it was observed, that the quality of life scores had not gone lower than the baseline scores as in the case of Group.1 and 2. The reason could be the reduction in the frequency of ADL attacks that were reported and the

TABLE 1.1

Change in Clinical parameters of the LE legs in three groups at intake & 12 months follow-up

Clinical Parameter	Group 1		Group 2		Group 3	
	Intake (n = 67)	12 months follow-up (n = 61)	Intake (n = 63)	12 months follow-up (n = 57)	Intake (n = 66)	12 months follow-up (n = 63)
	No.+ve (%)	No.+ve (%)	No.+ve (%)	No.+ve (%)	No.+ve (%)	No.+ve (%)
Skin Colour	30 (44.78)	18 (29.50)	29 (46.03)	17 (29.82)	40 (60.61)	28 (44.44)
Skin Texture	40 (59.70)	30 (49.18)	35 (55.56)	25 (43.85)	53 (80.30)	44 (69.84)
Ulcer	9 (13.43)	7 (11.47)	4 (6.35)	2 (3.50)	6 (9.09)	9 (14.28)
Wart	5 (7.46)	6 (9.83)	1 (1.59)	1 (1.75)	4 (6.06)	3 (4.76)
Intertrigo	18 (26.87)	16 (26.22)	13 (20.63)	10 (17.54)	22 (33.33)	15 (23.80)

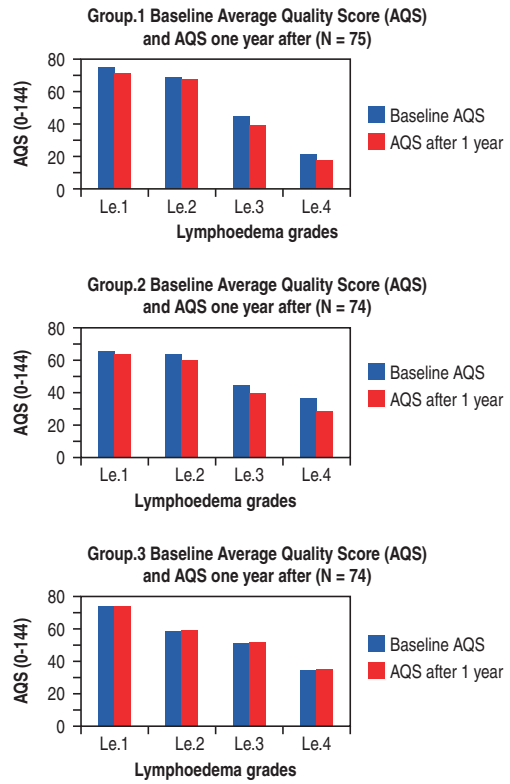


Figure 1.1 Impact of Morbidity Management and Disability Prevention (MMDP) on the Health Related Quality of Life (HRQOL) of LF patients.

small signs of change reportedly experienced by patients, in the form of slight reduction in pain and heaviness of oedema, and improvement in skin texture. HRQoL scores of Group 3 patients indicates that, regular practice of leg hygiene along with supportive treatment to reduce inflammation, pain and lesions, may lessen the suffering and improve the quality of life of LF patients.

Data analysis is being carried out taking LE leg as the unit. Recoding of the clinical scores will be done and analysed using SPSS software.

1.1.2. 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 monitoring and evaluation of lymphatic filariasis (LF) has largely relied on the detection of antigenemia and antibodies in human populations. Molecular xenomonitoring (MX), the detection of parasite DNA/RNA in mosquitoes, may be an effective complementary method, particularly for detecting signals in low-level prevalence areas where *Culex* is the primary mosquito vector. This study investigated the application of a household-based sampling method for MX in one of the PHCs in Thanjavur district, Tamil Nadu, India.

Objectives:

- To evaluate a mosquito collection sampling strategy that can be used to assess the usefulness of vector infection monitoring by PCR as a surveillance tool for assessing post MDA situation
- To assess the risk of resurgence of LF seven years after stopping MDA (long term impact) in the study area

MX surveys were conducted in 2010 in two evaluation units (EUs): 1) a hotspot area with higher LF prevalence in humans, and 2) a larger area that also encompassed the hotspots. Households were systematically selected using a sampling interval proportional to the number of households in the EU. Two independent samples were taken in each EU to assess reproducibility of results. Follow-up surveys were conducted in 2012 (4-years post-MDA) and in 2015 (7-years post-MDA). Mosquito pools were collected and analyzed by real-time polymerase chain reaction (qPCR). Filarial parasite DNA was extracted using two extraction procedures during 2010 and 2012: (i) commercially available qiagen kits and (ii) VCRC developed TE method and in 2015, only VCRC-TE extraction procedure was used, as the results of VCRC-TE extraction were found to be comparable to that of qiagen method (see Section iii below).

i. *Evaluation of sampling methodology:* The sampling methodology was evaluated by analyzing the data generated during 2010 and 2012. In 2010, the proportion of positive pools in the hotspot EU was 49.3% compared to 23.4% in the overall EU. In 2012, pool positivity was significantly reduced to 24.3% and 6.5%, respectively ($p < 0.0001$). Pool positivity based on independent samples taken from each EU in 2010 and 2012 were not significantly different except for the hotspot EU in 2012 ($p = 0.009$). The estimated prevalence of infection, measured by PoolScreen, declined from 2.2–2.7% in 2010 to 0.6–1.2% in 2012 in the hotspot

area and from 0.9–1.1% to 0.2–0.3% in the larger area.

ii. *Simulation of sampling strategies:* The data generated in 2010 and 2012 were used to optimize the sampling strategies. Two sampling plans were simulated to assess whether sampling effort (sampling 300 households (HH) x 1 pool per HH instead of 150 HHs x 2 pools per HH) could be minimized without loss of statistical power. The results showed that both the pool positivity and the vector infection rates were comparable between the two plans (Figure 1.2). Further, it was found that only in less than 2% of the simulations both the results differ significantly between plans.

iii. *Comparison of DNA extraction methods:* Figure 1.3 shows the results of qPCR assay based on DNA extraction by qiagen and VCRC-TE methods for the survey during 2010 and 2012 in both PHC areas and 'hotpots'. The rates were comparable between the two DNA extraction methods for both 2010 and 2012 (P>0.05) in the PHC areas. A similar observation could be made in the 'hotspots' except during survey 1 in 2012 in the 'hotpots'. Further, comparison of the vector infection rates showed that the rates did not differ significantly between the two DNA extraction methods over different surveys in both PHC and 'hotspots' during 2010 and 2012 (Table 1.2, 95% CI, overlap between the methods). Since the VCRC-TE extraction procedure yielded comparable estimates of vector infection rates, the qPCR assays in 2015 were restricted to VCRC-TE method of DNA extraction.

iv. *Long term impact of MDA on the risk of resurgence of LF:* In 2015, MX surveys were carried out to assess its usefulness for tracking long term (7-years post-MDA) changes in vector infection following 8 annual rounds of MDA. Both the pool-positivity and vector infection rates declined significantly with 2, 4 and 7-years post-MDA in the PHC areas as well as in the 'hotspots'. However, both rates were significantly higher in the 'hotspots' than that in the PHC areas (Figure 1.4).

Conclusions:

- A sample of 300 pools by collecting 2 pools of 25 mosquitoes each from 150 households could be used to reduce the sampling

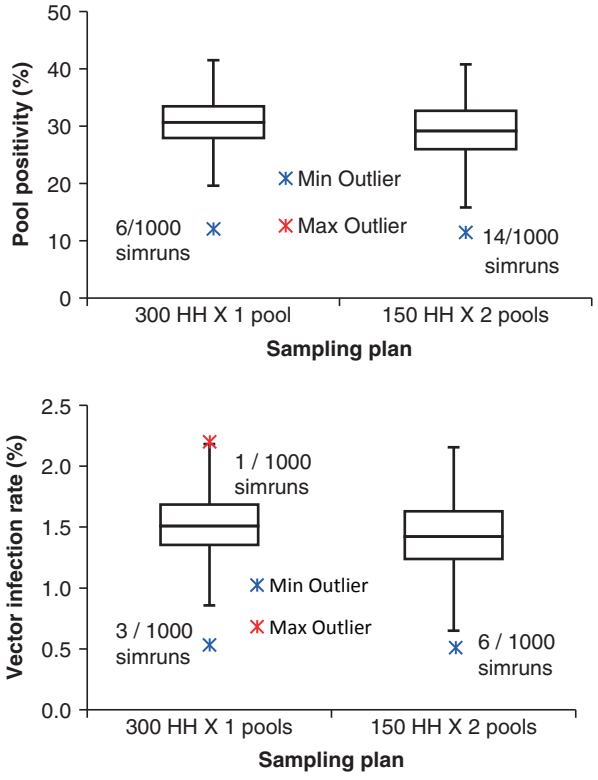


Figure 1.2 Comparison of simulated sampling plans by pool positivity and vector infection rates using data from all the 'PHC clusters'

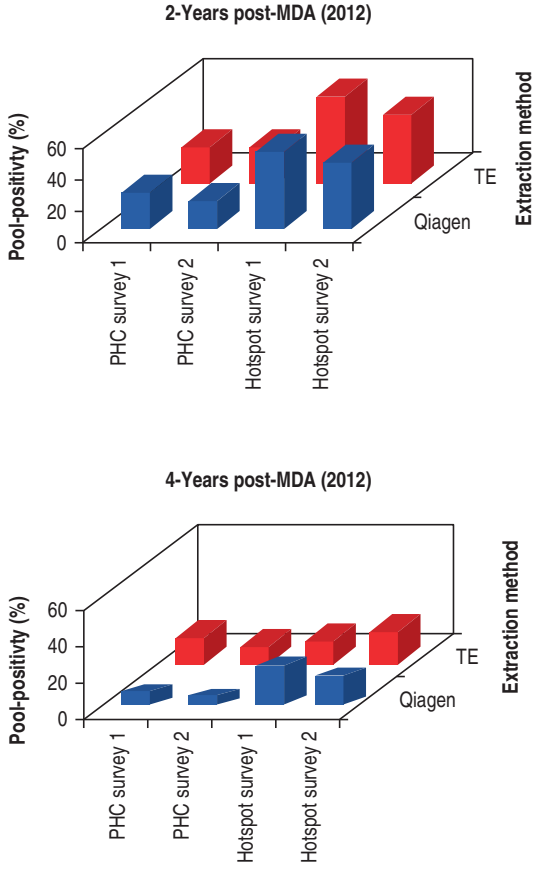


Figure 1.3 Comparison of pool positivity (%) between two DNA extractions methods in the PHC areas and in 'hotspots' during 2- and 4-years post-MDA (i.e. 2010 and 2012)

TABLE 1.2

Comparison of vector infection rates between two DNA extraction methods (qigaen vs VCRC-TE)

Stage	Survey area	# clusters	Qiagen method		VCRC-TE method	
			# pools processed	Infection rate (%, 95%CI)	# pools processed	Infection rate (%, 95%CI)
Stage 1	PHC survey 1	33	231	1.13 (0.82-1.51)	231	1.09 (0.79-1.46)
	PHC survey 2	33	230	0.85 (0.58-1.18)	224	1.06 (0.76-1.43)
	Hotspot survey 1	17	207	2.67 (2.12-3.32)	207	2.67 (2.12-3.32)
	Hotspot survey 2	17	207	2.24 (1.74-2.82)	207	2.24 (1.74-2.82)
Stage 2	PHC survey 1	33	231	0.29 (0.15-0.49)	230	0.68 (0.45-0.97)
	PHC survey 2	33	231	0.24 (0.12-0.43)	231	0.46 (0.28-0.72)
	Hotspot survey 1	17	206	1.17 (0.83-1.58)	207	0.57 (0.36-0.86)
	Hotspot survey 2	17	206	0.63 (0.40-0.93)	206	0.83 (0.56-1.18)

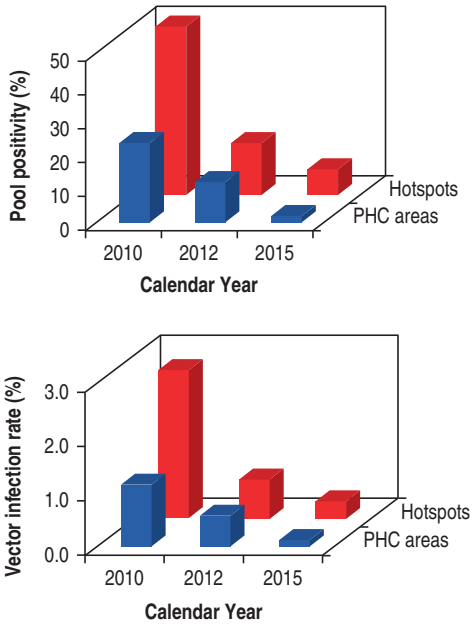


Figure 1.4 Long term impact of MDA on the risk of resurgence of LF measured by pool positivity and vector infection rate

effort instead of one pool from each of 210 or 300 households respectively.

- The household-based sampling strategy for MX led to reproducible results and supported observed trends in LF infection in humans.
- qPCR assay results of VCRC-TE based DNA extraction method are comparable to that of commercially available qiagen method, leads to cost reduction.
- MX has the potential to be a low-cost, non-invasive monitoring and evaluation tool with sensitive detection of infection signals in low prevalence settings.
- Further investigation and application of this sampling strategy for MX are recommended to support its adoption as a standardized method for global LF elimination programs.

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

IM 1501: Feb 2015 – Jan 2017

Vasuki, V & Nandakumar, Y

Development of an indigenous antigen based assay would serve as a valuable and additional diagnostic tool in Transmission Assessment Survey (TAS) for making decision on stopping MDA and Post-MDA surveillance until certification of elimination of LF. Cuticular collagen 2 (*col* 2) encoding genes of *Wuchereria bancrofti* are highly immunogenic and potential therapeutic candidates for blocking transmission. In the present study, the antigenic determinants of the cuticular collagen 2 protein of *W. bancrofti* were identified and assessed for their immunogenicity for the specific diagnosis of *W. bancrofti* infected individuals towards developing an antigen assay.

Objectives:

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

Among the four peptides designed (CCP1, CCP2, CCP3, CCP4), synthesized and evaluated, two of them showed immunogenicity when titrated individually (Annual Report, 2015). In order to maximize the immunogenicity, peptides were tested in cocktail form (as per the recommendation of the SAC, 2015) at three concentrations (0.4, 0.2 & 0.1 µg), with different combinations. Among these, the combination of CCP2 and CCP4 at 0.2 µg showed immunoreactivity in terms of OD value **0.864** (0.181). Based on the results, combinational polyclonal anti-peptide antibody (for CCP2 and CCP4) generation in rabbits was outsourced. Rabbits were immunized with 250 µg of each peptide conjugated with KLH in 0.5 ml PBS mixed in 1:1 with complete Freund's adjuvant and further boosted with same concentration as given earlier with incomplete Freund's adjuvant at 2nd, 4th & 6th week after immunization. Rabbit blood was collected 5 days after the last immunization, serum separated and titer estimated by ELISA using Bovine serum albumin (BSA) conjugated peptides. The titre of these peptides was found to be > 40,000 dilution with respective OD of 1.65 and 1.74 for CCP2 and CCP4 (Figure 1.5).

Purified polyclonal (designated as CCEP2/CCEP4) HRP-conjugated antibodies were coated on the plate surface and upon blocking with BSA, different dilutions of known positive and negative controls were tested by indirect ELISA. Dose-dependent response was found in respect to dilution among the tested sample, however, no significant difference was observed between positive and negative samples (Figure 1.6). When the test was repeated with sandwich ELISA format, development of high background was obtained. When Dot-blot was performed to identify the problem, same intensity in the colour development was observed in the positive and negative samples (Figure 1.7). To address this issue, different blocking solutions (BSA, Skimmed milk & Gelatine) were used for the Dot-blot and signal development was observed in negative control (Figure 1.8). The possibility of signal development in Dot-blot due to human albumin was also ruled out (Figure 1.9). The unconjugated peptides showed good signal in dot blot experiments which indicate that the antibodies are specific to CCEP2/CCEP4. Since the antibodies are polyclonal in nature, it is difficult to assess the non-specific interaction with negative controls. To overcome this problem, isolation of antibodies specific to CCEP2 and CCEP4 from the serum will be carried out by using antigen affinity purification to develop ELISA using these

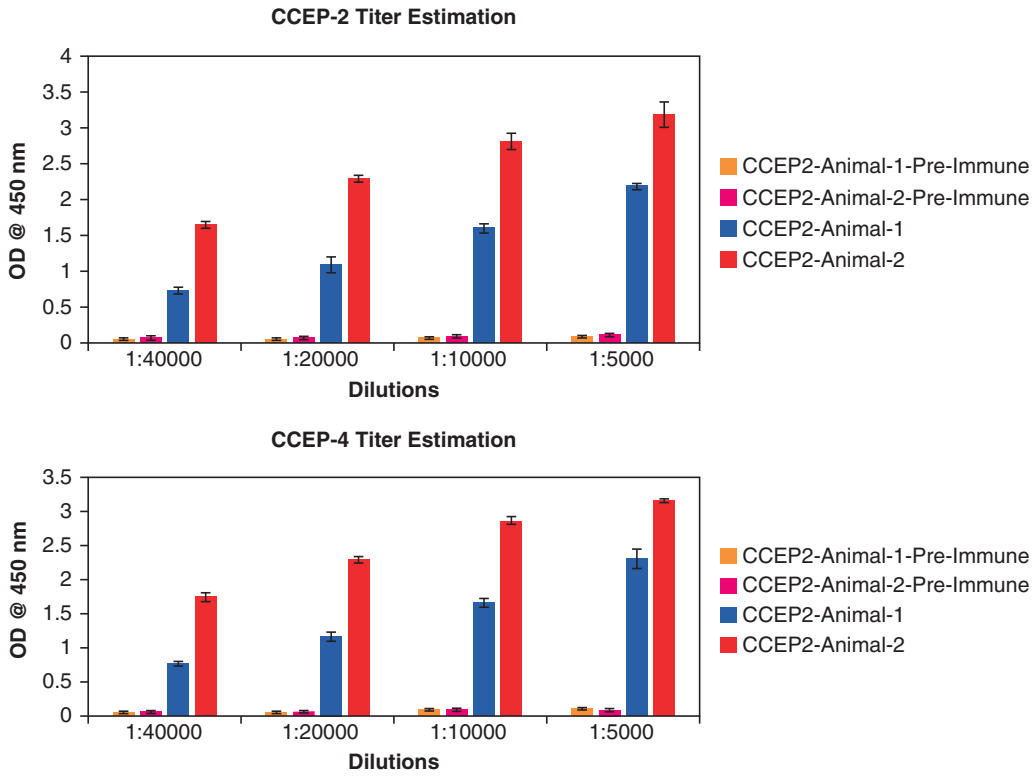


Figure 1.5 CCEP-2 Titer Estimation & CCEP-4 Toter Estimation

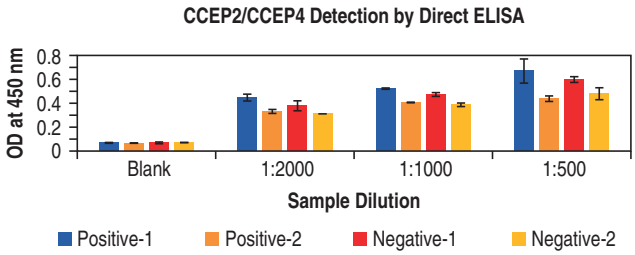


Figure 1.6 CCEP 2 / CCEP 4 Detection by Direct ELISA

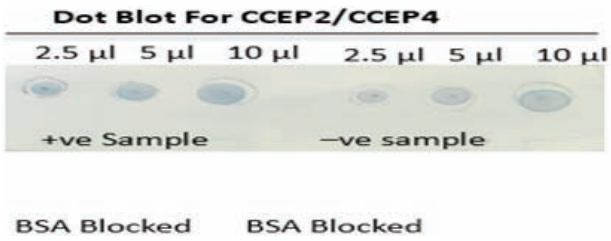


Figure 1.7 Dot Blot experiment with positive and negative samples with blocking solution BSA

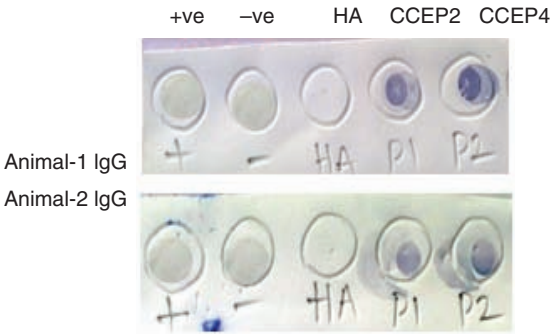


Figure 1.8 Dot Blot with Human Albumin

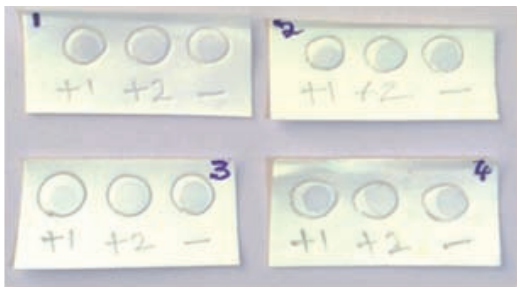


Figure 1.9 Dot Blot with Gelatin and Skim milk

antibodies Recombinant expression of CCEP for positive control to develop and validate the assay and Monoclonal antibody generation to get highly sensitive and specific antibodies to CCEP through outsourcing is in progress.

1.1.4. District level validation of xenomonitoring of infection in Culex vector by PCR as a surveillance tool for lymphatic filariasis elimination programme

IM 1403: Jun 2014 – Dec 2016

Subramanian S[1], Krishnamoorthy K[1], Sadanandane C[1], Vasuki V[1], N. Sivaganame[1], C. Palaniswamy[2], and Jambulingam P[1]
[1]Vector Control Research Centre, Pondicherry;
[2]Office of the Deputy Director of Health Services, Department of Public Health, Cuddalore, Tamil Nadu

The WHO recommended strategy for making decision to stop / continue the mass drug administration (MDA) programme and post-MDA surveillance is the transmission assessment survey (TAS). TAS is based on detecting filarial antigen in children (age-group: 6–7 years) using ICT / filariasis test strip (FTS). As an alternative to detecting infection in human, infection in vectors could be monitored when human infection levels are very low following the cessation of MDA. The challenge in using vector infection as an indicator for making decision on stopping MDA or post-MDA surveillance is to establish / set a threshold level for vector infection. The VCRC has developed and tested a two-stage cluster design based sampling strategy for collecting vector mosquitoes and monitoring vector infection by PCR assay at sub-district level. Also, it was estimated that a vector infection level of 0.6% was equivalent to 2% Ag-prevalence in children of 6–7 years in children, the recommended cut-off value for stopping MDA using TAS. This study, using the cut-off value, validates the usefulness / feasibility of xenomonitoring 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

Cuddalore district (implementation unit, IU), in Tamil Nadu is the study area. The IU has undergone 12 rounds of mass drug administration (MDA) since 1996–97. Since the total population

(2.4 million) of the IU is above 2 million, it was divided into two evaluation units (EU1 and EU2) as per the WHO protocol for conducting TAS. Of the two EUs, EU1 was selected for the study, which consists of four taluks (population: 1.4 million): (i) Cuddalore, (ii) Chidambaram, (iii) Kurinchipadi and (iv) Pantruti.

The two-stage cluster sampling protocol, which was developed and validated at sub-district level for sampling *Culex* mosquitoes and molecular xenomonitoring of filarial infection was adopted. A detailed description of the sampling methodology is reported in VCRC Annual Report 2015. Briefly, the methodology involves (i) selection of 30 villages / wards (clusters, stage 1) from the list of clusters in the EU and (ii) selecting an average of 5 households (stage 2) per cluster and from each selected household 2 pools each of 25 gravid *Culex* female mosquitoes were collected by placing gravid traps for a maximum 3 nights.

A total of 346 pools (comprised of 8750 of *Culex* gravids) collected from 186 households spread over 30 clusters in the EU were subjected to quantitative PCR assay. Of them 9 pools (2.6%) were found to be positive for filarial infection. The prevalence of infection was found to be 0.11% with 95% confidence limits of 0.05 to 0.21% (maximum likelihood estimate based on Poolscreen software ver. 2.0). The estimated infection level is much lower than the critical cut-off of 0.6%. The result confirms the TAS (4 Ag-positive children < critical cut-off of 18) based decision to stop MDA in the EU.

In order to have a parallel assessment of vector infection with Mf-prevalence, microfilaria survey is in progress in all the 30 sites where xenomonitoring was done. So far a total of 3500 persons from 6 clusters were screened for Mf and none were found positive for Mf.

1.1.5. 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 is targeted for elimination as a public health problem by 2020. In India elimination was aimed to achieve by 2015. However, due to operational issues related to achieving the targeted compliance in some of the 'hard core' districts, there are indications that the programme in India requires to be extended beyond 2015. Persistence of infection even after 10 rounds of MDA in some of the implementation units (IU) is a major challenge to the programme. This study aimed to help in (i) predicting the scope of accelerating the LF-elimination programme with strategy adjustments by considering 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 strategies for monitoring epidemiological situation after cessation of 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.

The LYMFASIM simulation model has been adapted for India by extending the model outcomes to predict the prevalence of antigenemia (Ag) as detected by the immunochromatographic card (ICT) (Objective 1). Further the model was used to estimate the required duration of MDA with the 2-drug regimen (DEC plus albdendazole) for different endemic settings (Objective 2), and the 1-year post-MDA infection levels associated with successful elimination (Annual Report 2015). During the reporting period, LYMFASIM was used to simulate and compare the number of rounds of MDA required to achieve a microfilaremia prevalence of <1% with a single dose of 3-drug regimen (ivermectin, diethylcarbamazine, and albendazole, IDA) against the currently used 2-drug MDA (DA) regimen for accelerating LF elimination in the 'hard core' districts, where the prevalence of Mf is ~5%. The results of LYMFASIM were compared with that of two other models developed by other partners of the NTD modelling Consortium (TRANSFIL by Warwick University, UK and EPIFIL, by Notre Dame University, USA). LYMFASIM and TRANSFIL

are based on the technique of stochastic micro-simulation (individual based) and EPIFIL is a deterministic macro-simulation model (population based). In simulating the impact of IDA, the following assumptions were made about the efficacy of IDA:

- IDA1: the same macrofilaricidal properties as ALB+DEC (55%) and the remaining worms are permanently sterilised (100%).
- IDA2: the same macrofilaricidal properties as with the ALB+DEC regimen (55%).
- DA and IDA were assumed to have a microfilariacidal effect of 95% and 100% respectively.

Figure 1.10 shows the probability of reaching <1% Mf-prevalence in a single round of MDA with IDA for varying levels of coverage in epidemiological different settings (~5%, 10% and 15%). The prevalence in the 'hard core' districts with the existing target of 65% coverage is unlikely to reduce the rate to below 1% in a single round (Figure 1.10). At a prevalence of 5%, the overlap between those individuals who are infected and those who are treated at a coverage of 60% may be small, resulting in too small a reduction in prevalence (Figure 1.10, inset). As coverage increases, the probability of reducing Mf rates to <1% increases markedly, but is only over 80% when coverage exceeds 80%. In comparison, the counterfactual DEC+ALB regimen had negligible probability of (<0·1%) of reducing prevalence to target levels for any coverage.

Figure 1.11 shows the probability of reaching the pre-TAS threshold Mf-prevalence of 1% for varying levels of coverage in the 'hard core' districts. The probability of achieving the <1% Mf prevalence target is moderately high, even for the two-drug regimen. For three rounds, summarising across models, the probability of achieving the target is 48% compared to a 95% probability with IDA1 (Figure 1.11). However, once the number of rounds increases to five (the current standard protocol), the difference between the regimens is small (95% standard regimen to 100% IDA1). IDA reduced the number of rounds of MDA required to reach the target by 1 or two rounds when the baseline prevalence was ~5%; it also reduced the probability that large numbers of rounds are needed by chance.

Changing coverage has a strong impact in reducing the rounds required to achieve less than 1% Mf prevalence in the 'hard core' districts for both the IDA and ALB+DEC regimen (Figure 1.12). Increasing coverage from 65% to 75% decreases the number of rounds required to have a 95% probability of reaching the target prevalence of

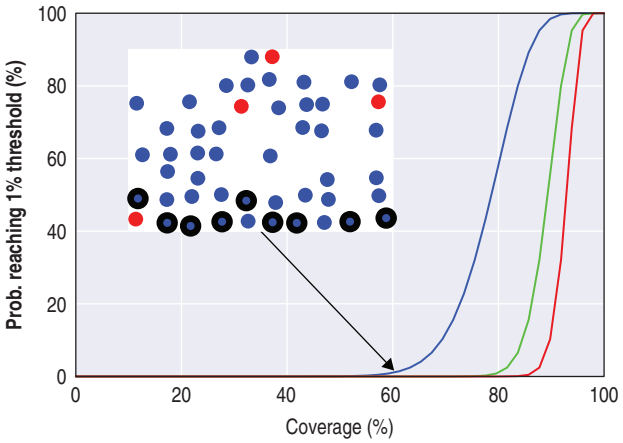


Figure 1.10 The probability that a single round of MDA results in prevalence falling below 1% for IDA. Results are shown for initial prevalences of 5% (blue), 10% (green) and 15% (red). Inset: illustration of setting with 5% prevalence (red spots) and 95% uninfected (blue spots), and a 65% coverage (black circles), resulting in only a small probability of prevalence falling below 1%.

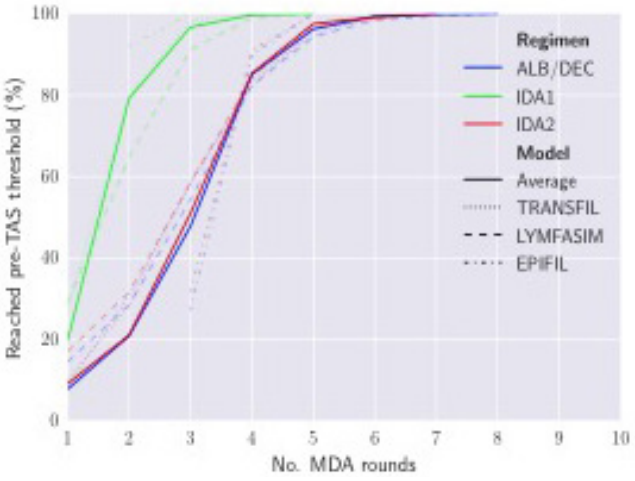


Figure 1.11 The impact of the IDA regimen in 'hardcore' districts with ~5% Mf-prevalence. Solid lines indicate a model-averaged estimate and dashed lines are estimates from the individual models

1% from 7 to 3 rounds and from 3 to 2 rounds for Alb/DEC & IDA1 respectively. Systematic non-adherence has a dramatic impact on the outcome of an MDA intervention at poor coverage (55%), increasing the rounds to achieve a 95% pass rate from 7 to 10 rounds and 4 to 8 rounds (Alb/DEC and IDA1 respectively). However at higher coverage (75%) the impact is less severe (rounds required for 95% pass rate increases by one for both Alb/DEC and IDA1).

Conclusion

Simulation modelling suggests that IDA has the potential to accelerate the elimination of lymphatic filariasis if high coverage (70%) of MDA can be achieved and if rates of systematic non-adherence with MDA are low.

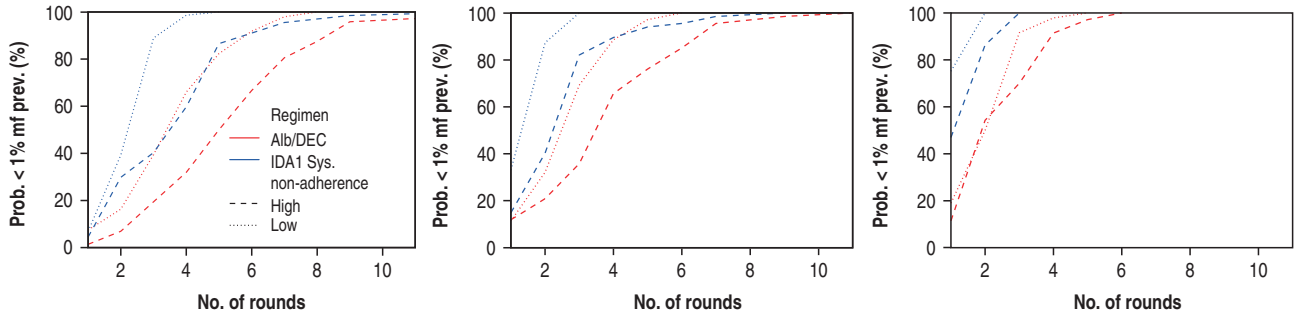


Figure 1.12 Relative impact on coverage and systematic non-adherence (Low: a randomly chosen individual's decision on adhering to MDA was the same as their decision in the previous round 25% of the time; High:75% of the time) on the probability of reaching <1% Mf-prevalence.

1.1.6. Development and demonstration of strategies to enhance community compliance for MDA towards Lymphatic filariasis elimination in Palakkad district of Kerala state

EM 1511: Jan 2015 – Dec 2016

Nandha B, Vijayakumar KN, Meenakshy V, NVBDCP Kerala

LF was a major public health problem in the state of Kerala with 11 endemic districts out of 14 districts. All the 11 districts were covered under MDA since 2004 and four districts have qualified for stopping MDA, another four districts qualified for Transmission Assessment Survey (TAS) and MDA is continued in the remaining 3 districts. Among these 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. At the request of the Directorate of Health Services, Kerala, a study was initiated to identify the causes for the low drug consumption, develop and demonstrate appropriate strategies to improve drug coverage and accelerate compliance to MDA.

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

A three arm intervention study has been implemented in 21 sites in Palakkad district to improve drug coverage and compliance.

Following the intervention, the performance in preparing the programme staff in drug distribution and logistics improved. The gap noticed was bridged except timely distribution of IEC materials. Quantitative and qualitative surveys were carried out in 21 sites (4 wards of 2 municipalities and one Sub centre area from 17 PHCs). Detailed qualitative interviews were conducted with 676 and 691individuals and information on consumption was gathered for 2298 and 2563 individuals in the pre intervention and post intervention surveys respectively. The results indicated that there was a significant improvement in post intervention MDA in coverage of drug distribution and consumption in all the three arms. A significant improvement was also observed in coverage and consumption in Arm I (where additional inputs have been executed) and Arm III (Programme only) (Figure 1.13). The difference in odds ratio shows that the relative change in consumption is significantly higher in the intervention arm (Arm I)

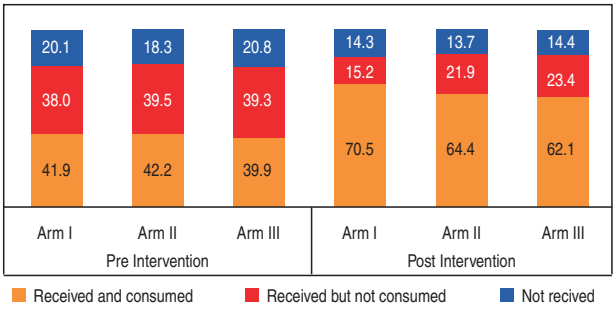


Figure 1.13 Coverage of distribution and compliance to MDA pre and post intervention in the three arms

TABLE 1.3

Pre vs post intervention coverage, compliance and Mf prevalence

Intervention	No. Study sites	Pre-intervention						Post-intervention					
		n	Cover-age	Consump-tion	Compli-ance	Mf prevalance		*n*	Cover-age	Consump-tion	Compli-ance	Mf prevalance	
					%	*n*	%				%	*n*	%
Arm I Additional inputs from VCRC	7	874	678 (79.9)	366 (41.9)	**52.4**	1493	1.55	892	765 (85.8)	629 (70.5)	82.2	1476	0.88
Arm II Programme with VCRC support	7	765	625 (81.7)	323 (42.2)	**51.7**	1438	2.83	882	761 (86.3)	568 (64.4)	74.6	1481	1.55
Arm III Programme only	7	659	522 (79.2)	263 (39.9)	**50.4**	1515	1.36	789	675 (85.6)	490 (62.1)	72.6	1283	0.94
Total	**21**	**2298**	**1845 (82.4)**	**952 (39.9)**	**50.4**	**4446**	**1.90**	**2563**	**2201 (82.4)**	**1687 (65.8)**	**76.6**	**4240**	**1.13**

Figure in parenthesis denote percentage

compared to Arm II where strengthening of social mobilization with VCRC support was carried out and control arm (Arm III) reflecting the effectiveness of the intervention activities.

Although a reduction in Mf prevalence was observed in all the three arms, the reduction was significant in Arm I compared to Arm III and can be attributed to the higher levels of drug consumption (Table 1.3).

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

EM 1512: Nov 2015 – Oct 2017

Nandha B, Krishnamoorthy K, Sharma SN, NVBDCP
Collaborating Institutes/departments: NVBDCP Bihar, Surat & Telengana

India has made appreciable progress to achieve the national goal of elimination of lymphatic filariasis (LF) as a public health problem. Mass Drug Administration (MDA) programme was launched in 2004 to eliminate LF from all the 250 endemic districts in the country. In spite of 10–12 rounds of treatment, only 73 districts have qualified for stopping MDA and in 134 (53.6%) districts MDA is still continued. Bihar, Andhra Pradesh and Gujarat have 38, 16 and 11 endemic districts respectively with more than 1% Mf prevalence. This challenge requires careful review of both technical and operational aspects of the programme, such as the coverage and compliance of the MDA.

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

Intervention Mapping (IM), a stepwise approach for theory and evidence based development and implementation of interventions will be applied. A situation analysis to identify the gap and the determinants of the gap between coverage and compliance in MDA with anti-filarial drugs were carried out in 4 selected rural areas and 2 urban wards from Nalgonda and Surat districts of Telangana and Gujarat states respectively. The data collection in Muzzafferpur district of Bihar will be initiated.

Individual level determinants such as awareness, acceptance and attitude towards MDA and the reasons for non-compliance were assessed using in-depth interviews using Health Belief Model (HBM) with respondents from 360 households with 180 each per study district in Surat and Nalgonda districts. All the systematic non-compliers (not complied in any of the treatment rounds) are listed out from the selected households and are subjected to In-depth interviews using HBM. To assess community level determinants 24 Focus group discussions (FGD) and 24

Key Informants (KI) interviews were conducted in 2 districts. Drug distributors were interviewed for factors related to programme success and barriers using a questionnaire.

The MDA conducted in 2015 in each of the selected districts were assessed for estimated coverage and compliance using a standard format, based on the reported coverage provided by the concerned district authorities. The assessment was made in terms of proportion of people who have actually received DEC tablets (= coverage of drug distribution), those who have consumed the tablet (= consumption of tablet out of sampled population) and compliance (= consumption of tablet out of those received tablets) in the selected areas. In Surat district the estimated coverage was 86% and compliance was 60%, where as in Nalgonda and Muzzafferpur districts the coverage and consumption was below the optimum level (Table 1.4), clearly indicating the challenges in programme performance and social mobilization.

Except Surat, the coverage and consumption of MDA was found to be sub-optimal in the other two districts. Coverage is an indicator of programme performance and consumption is an indicator of social mobilization. On completion of data collection the changeable determinants will be identified and performance objectives will be derived. A brain storming session with the programme people will be conducted for practical solutions.

1.1.8. A community based study, to compare the safety, efficacy and acceptability of a triple drug regimen (Ivermectin, Diethylcarbamazine and Albendazole) with a two-drug regimen (Diethylcarbamazine and Albendazole) for lymphatic filariasis elimination programme

EM 1605: Sep 2016 – Dec 2017

Jambulingam P, Vijesh Sreedhar Kuttiat, De Britto RLJ, Subramanian S, Srivdya A, Nandha B and Krishnamoorthy K
Collaborating Institutes/Departments: National Vector Borne Diseases Control Programme, Delhi

In India the lymphatic filariasis (LF) elimination programme adopted the WHO recommended two-drug policy of co-administration of diethylcarbamazine (DEC) and albendazole (DA) in 2007 in all endemic districts and achieved success (microfilaria (mf)<1%) in most districts. However, mf levels remained >1% in 31 "hard-core" foci (districts) and the national programme was looking for additional tools to accelerate interruption of transmission in these districts to achieve LF elimination by 2020. Meanwhile, the results of a clinical trial conducted in Papua New Guinea (PNG) during 2015 showed that triple drug therapy (ivermectin, DEC, albendazole (IDA)) is superior to the current two-drug regimen in terms of sustainable reduction of mf (zero at 1 year) and safety profile.

TABLE 1.4

Assessment of MDA compliance – 2015

State	PHC	Village/ward	No. Sampled	Received		Consumed		Compliance %
				No.	%	No.	%	
Muzzafferpur	Municipality	Kiranpatti	180	75	41.67	28	15.56	37.33
	Bochaha	Bochaha	212	62	29.25	16	7.55	25.81
	Kanti	Akuhara	181	118	65.19	61	33.70	51.69
	Motipur	Pachrukhi	174	72	41.38	17	9.77	23.61
	Total		**747**	**327**	**43.78**	**122**	**16.33**	**37.31**
Surat	North Zone	Katargam	168	154	91.67	82	48.81	53.25
	East Zone	Puna	144	113	78.47	78	54.17	69.03
	South east Zone	Goddhara	155	133	85.81	83	53.55	62.41
	Erthan	Erthan	164	143	87.20	133	81.10	93.01
	Total		**631**	**543**	**86.05**	**376**	**59.59**	**69.24**
Nalgonda	Peddavoora	Pinnavora	156	20	12.82	14	8.97	70.00
	Vemulapally	Vemulapally	153	86	56.21	22	14.38	25.58
	Ramalabanda	Ramalabanda	154	86	55.84	34	22.08	39.53
	Municipality	Ward 28	151	75	49.67	22	14.57	29.33
	Total		**614**	**267**	**43.49**	**92**	**14.98**	**34.46**

However, prior to implementing this three drug regimen, its safety, efficacy and acceptability profile needs to establish and this requires pre and post treatment assessment from at least 10,000 people treated across multiple settings (~4000 from India). It is therefore proposed to conduct a study to acquire safety, efficacy and acceptability data in India before the new IDA regimen can be used in MDAs.

Objectives:

- To determine the frequency, type and severity of adverse events following triple-drug therapy (IVM+DEC+ALB, IDA) compared to the standard two-drug treatment (DEC+ALB, DA) in infected and uninfected individuals in a community.
- To compare the efficacy of IDA vs. DA administered in communities for clearance of Mf and filarial antigenemia (Ag) in cohort and effectiveness (prevalence) in community settings.
- To assess the presence and intensity of filarial infection on the frequency and severity of adverse events.
- To compare community acceptance of MDA with IDA vs. DA.

Implementation: Two blocks each with 2 villages, comparable with respect to microfilaria (mf) rate and population size were selected for the study. The blocks were randomized for allocation of the treatment regimens: the block with villages Hattikunni and Gunjanur were selected for three drug arm (Ivermectin, Diethylcarbamazine and Albendazole (IDA)) and other one with Gajarkote and Khandkur were allocated for the two drug (Diethylcarbamazine and Albendazole (DA)) arm. Currently, the enrolment is carried out only in Hattikunni village (IDA arm).

The social scientists and workers visited the village 1-2 days prior to implementation and preparation of the community for the study. They identified the list of households to be visited by the teams for enrolment. Each team consists of a MO, JN, DEO and 3 technicians (total 6 members). The teams visited the households identified by the social workers, the MO/JN explained the details of the study to the eligible participant and obtained informed consent. Clinical history of the participant was elicited by the MO and entered in the EDC system by the DEO. Following this, the technicians carried out the FTS test and the results at 10 minutes were conveyed to the DEO to be entered in the EDC. Those with negative FTS results were administered the three drug regimen by the MO/JN. Those having positive results for FTS were visited again in the night after 8.30 pm and after taking blood smears from them, drugs were administered. The entire activities of enrolment and drug administration were carried out between 6 pm and 10 pm when majority of the community members were available in the village.

Adverse Event/Serious Adverse Event Monitoring: Monitoring for adverse events (AE) was done from day of enrolment (day 0) itself by a clinician, who camped in the PHC, within the village for the night, to provide on-site management of adverse events, if any. Active monitoring was carried out on days 1 and 2 by the team (clinician, junior nurse and DEO – who enrolled) visiting the treated households and recording AEs, if any. A passive monitoring team (clinician, junior nurse and DEO) were stationed at the PHC between days 3 and 7, to treat those reporting to PHC with AEs. Details of AEs were entered in the EDC by the DEO.

Progress: A total of 8 teams were formed and participant enrolment commenced in Huttikunni, the village under the IDA arm on 7th October 2016. As of 31st October 2016, 7 days of enrolments have been completed. A total of 634 subjects consented for participation of whom 577 (91%) were administered IDA (Table 1.5). Others refused.

Among those consumed the drugs, 47 (8.1%) developed AEs of which 45 (7.8%) and 14 (2.4%) presented with Grade1 (mild) and Grade2 AEs (moderate). None reported with SAEs. All AEs were managed by providing treatment.

TABLE 1.5

Details on enrolment, administration of IDA and adverse events among those enrolled

Arm	Number given informed consent	Number administered IDA	Number of persons with AEs	Number of adverse events	Number of Grade 1 events	Number of Grade 2 events
IDA	634	577	47	80	66	14

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

EM 1606: Oct 2016 – Sep 2018

Vasuki V, Sadanandane C, Subramanian S, Natarajan R, Sivagnanam N, Jambulingam P, Chief Entomologist, DPH & PM and a Senior Entomologist from the Zonal Entomological Team from the respective district / State

Monitoring and epidemiological assessment of mass drug administration (MDA) and post MDA surveillance are important issues for the LF elimination programmes. Programme success or failure can be monitored by measuring changes in infection status in either the human or vector. TAS is designed and recommended by WHO to be a practical and effective evaluation tool for making decision on stopping MDA (WHO, 2011), although its validity for longer-term post-MDA surveillance requires further investigation. Recent study in Sri Lanka revealed that TAS alone may not be sufficient for assessing the success of filariasis elimination program and also recommended the use of xenomonitoring of vector infection as one of the tools to complement TAS for post MDA surveillance. The Vector Control Research Centre has developed a two-stage cluster design based sampling strategy for sampling vector mosquitoes using gravid traps and xenomonitoring of vector infection by PCR assay that could aid programme managers for making decision about stopping MDA or post MDA surveillance. This project is to validate the usefulness of xenomonitoring as an alternative to TAS during post MDA surveillance in operational settings (in three districts) so that it can be translated into the elimination programme.

Objectives:

- To assess the usefulness of xenomonitoring in the following post-MDA districts as an alternative to Transmission Assessment Survey (TAS) and its cost-effectiveness

Three districts selected for the study are categorized as (i) awaiting for TAS/TAS failed (Kurda dt in Odisha), (ii) TAS I passed (Cuddalore Dt in Tamil Nadu) and TAS II passed (Pondicherry in Pondicherry UT). In all the three categories of the districts, (i) Mf survey, (ii) Transmission Assessment Survey (TAS) – Antigen survey and (iii)Vector survey for xenomonitoring will be carried out to assess the level of infection in human (Microfilaraemia and Ag) and in vector (infection and infectivity). First instalment funds received in October, 2016. Project staff recruitment is in progress.

1.2 LYMPHATIC FILARIASIS UNDER TRANSLATIONAL RESEARCH

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

EM 1305: Jun 2013 – May 2017

RMRC, Port Blair: Shriram AN, Vijayachari P
VCRC: Jambulingam P, Krishnamoorthy K,
Port Blair: Amitabha De, (Malaria, Filaria & VBDs),
Avijit Roy, DD (Health), Directorate of Health Services, A&N Administration

Nancowry group of islands comprises of 5 remotely located islands viz. Chowra, Kamorta, Katchal, Nancowry and Teressa, Nicobar district. Diurnally sub-periodic *Wuchereria bancrofti* (DspWB) is endemic to these islands, where it is transmitted by mosquito *Downsiomyia nivea*, a day biting mosquito. The programme to eliminate lymphatic filariasis through mass annual single dose of DEC with albendazole (ALB) was launched in 2004. Despite 12 rounds of MDA with DEC and albendazole, the infection remained > 1 %. The RMRC in collaboration with VCRC, ventured into the task of demonstrating elimination with DEC fortified salt as a supplementary measure in the DspWB endemic area. A partnership approach with commitment from different stake holders is followed, which envisages a platform for productive alliance between the ICMR, Tribal council/village council and the Directorate of Health Services under the aegis of the nodal agency, NVBDCP. This project aims at distributing double fortified salt (iodine and DEC) for one year and to assess the operational feasibility, community acceptance, efficacy and effectiveness of DEC salt distribution as a supplementary strategy to MDA. The overall objective is to demonstrate the usefulness of DEC salt in hastening the processes of achieving elimination of LF.

The project was initiated in 2013. Post initiation, the baseline activities included advocacy, enumeration of the population of the implementation unit, assessing the (baseline indicators) situation- microfilaraemia and antigenemia, assessing baseline vector infection rates and salt usage pattern. Implementation plan included stake holders meeting, establishing collaboration with Tamil Nadu Salt Corporation for quality assured double fortified salt, development of IEC tools and social mobilization strategies, delivery mechanism/strategy and concurrent monitoring of DEC salt consumption against the demand.

Base line data on *Mf* prevalence and Ag prevalence collected were used to form clusters based on the infection prevalence and population. Cluster randomization was followed and two islands were selected for each arm. Thus there are two arms in the study viz. the salt arm and MDA arm. There are 12 villages in the salt arm (Intervention: MDA + double fortified salt) (population = 3055) and MDA arm (Intervention: MDA alone) 14 villages (population = 4929).

The Chief Secretary and Health Secretary, A&N administration, launched the distribution of double fortified salt (DEC+Iodine) for the community at risk for the elimination of diurnally sub periodic filariasis in the Nancowry group of islands, on 20th November 2015. Subsequently, the Assistant Commissioner, Nancowrie launched the distribution of the double fortified salt at the implementation unit level and, Chief, Tribal Council released the IEC tools on 7th December 2015.

DEC powder was obtained from Syntholab, Mumbai authorized by the WHO. The technique of double fortification was standardized and Tamil Nadu Salt corporation supplied 25 metric tons of double fortified (DEC and iodine) free flow salt in 1 kg packs with message on the cover. Distribution of DEC fortified salt was commenced from December 2015 and the effective coverage (>85%) derived was achieved from January 2016 onwards. Distribution of double fortified salt (DEC+Iodine) for one year (Dec 2015–Dec 2016) in the intervention arm (Teressa and Nancowry) was completed. During this one year period a total of 20,981 (20.98 tonnes) of double fortified salt was distributed in the intervention arm for a population of 3055. The monthly coverage was >90% against the demand. Fortnightly household surveys showed that the target community used only fortified salt and >90% of the individuals used the double fortified salt for household purpose which was confirmed by the results of analysis of kitchen samples for DEC content. The double fortified salt is acceptable by the native Nicobarese community and is operationally feasible. DEC was found to be homogeneous and in the therapeutic range (0.2-0.32% w/w).

Impact assessment was done six months after the introduction of the salt distribution. A total of 4473 peripheral blood smears were collected from both the arms (Salt Arm: 2296 MDA arm: 2177).

In the salt arm the *Mf* prevalence was reduced from 2.1 to 0. 9%, whereas in the MDA arm, the overall *mf* prevalence is persistent at 1.8%. Efficacy of DEC fortified salt was assessed by testing a cohort of *Mf* positives. In the salt arm, out of the 45 microfilaria carriers monitored, the cure rate (complete clearance) was 81.3% while the success rate (number who showed reduced mf count) was 96.9%. In the MDA arm, out of the 38 microfilaria carriers followed, the cure rate was 63.3%, while the success rate was 70.0%.

The study is in progress and impact assessment on Mf prevalence and antigenemia among children will be carried out following one year intervention with DEC salt.

1.3 MALARIA / LEISHMANIASIS

1.3.1. 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 2013 – Oct 2016

Jambulingam P, Gunasekaran K, Sahu SS, Subramanian S, Behera S and Swati Kumari* (*State Health Dept.)*
Collaborating Institutes/Departments: Office of the Chief District Medical Officer, Koraput, Odisha

Integrated vector management (IVM) in National Vector Borne Disease Control Programme (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. However, 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 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 areas where the State NVBDCP has already been implementing vector control intervention measures. The study has three arms. Arm 1 includes 86 villages under eight sub-centres (SCs) with a population of 32,966 in Laxmipur CHC. Under Arm 2, 88 villages with a population of 33,969 spread in eight SCs of the same CHC are included. Arm 3 has 99 villages of seven 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, LLIN and IRS.

The study has three phases: 1. Base-line data collection with ongoing intervention measures (November 2013 to October 2014), 2. Input from VCRC on IEC activities for enhancement of coverage/compliance to interventions (November 2014 to October 2015) and 3. Reinforced IEC activities by VCRC (November 2015 to October 2016). Implementation of vector control measures by the State NVBDCP and the IEC activities by VCRC is common to all the three arms. Monitoring of entomological (density, survival and human blood index) and parasitological (parasite incidence) parameters was done in the index villages of the three arms (Arm 1: 8 villages, Arm 2: 8 villages & Arm 3: 7 villages) to assess the impact of the interventions. Also, operational evaluation of the two intervention measures was carried out in the three arms. The results of Phase 1 and 2 of the study have already been communicated (Annual Report 2014 & 2015) and the current report includes the results pertaining to Phase 3.

Spray coverage: The spray coverage during the Phase 1 (2013–14) in Arm 1 and 3 is shown in Figure 1.14. During Phase 2 (2014–15), 1st round spray coverage was 55.0% (range: 35.5%–64.9%) in Arm 1 and 55.5% (range: 52.3%–59.3%) in Arm 3; during the 2nd round, with VCRC input on

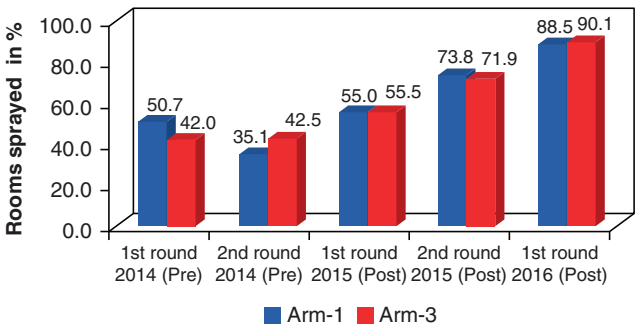


Figure 1.14 Indoor residual spraying coverage in Arm 1 & Arm 3 during Phase 1 (Pre) and Phase 2 & 3 (post)

IEC activities such as fixing 750 flex sheets on the main thoroughfare of the villages depicting importance of spraying for prevention of malaria and sending advance information to the villages about spraying and spray dates, coverage was improved to 73.8% (range: 63.8%–90.7%) in Arm 1 and 71.9% (range: 56.9%–80.5%) in Arm 3. During Phase 3 (2015–16), prior to 1st round of spraying IEC activities were strengthened; group meetings (Figure 1.15) and rallies were conducted in all villages and pamphlets/booklets were distributed to all schools spreading messages on value of spraying and people's cooperation with spray teams, and spray dates, necessity of spraying all rooms in houses and advantage of deferring mud-plastering the sprayed walls at least up to 3 months. As a result, the spray coverage during the first round in 2016 was further increased to 88.5% (range: 80.0%–98.1%) in arm 1 and 90.1% (range: 82.9%–98.3%) in arm 3 index villages.

LLIN usage and status: The net use rate 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 to 55.0% and 46.5% to 50.0%. There was no improvement in use rate during Phase 2 compared to Phase 1 even with the input on IEC by VCRC, mainly because of the fact that only around 50% of the distributed LLINs was in usable condition and rest were either damaged or missing as per the survey done by VCRC in May 2015. This gap was filled by distributing fresh insecticide treated nets (ITNs). The replenishment of nets coupled with reinforced IEC activities by VCRC, the net use rate was enhanced to 74.1% (range: 67.3%–80.2%) in Arm 1 and 73.8% (range: 58.9%–84.4%) in Arm 2 (Figure 1.16).

Cone bio-assay for insecticide effect of LLINs: Since adequate number of *An. fluviatilis* could not be collected in the study area, *An. jeyporiensis*, another susceptible species, was used for cone bio-assays. After 30 months of net distribution, cone-bioassay was done on 16 LLINs drawn from 16 randomly selected villages (one net from each village); the mosquito mortality was 97.5% (n=750). Cone bio-assay after 36 months of distribution showed only 77.5% (n=400) mortality. Since, the corrected mortality was < 80%, all the nets were treated with deltamethrin. After six months of use, the treated nets caused 100% mosquito mortality (n=400) in cone-bioassays on 16 ITNs, one net from each of the 16 index villages. Bio-assay after eight months of use also showed 100.0% mortality.

Cone bio-assay on sprayed surfaces for insecticide effect: Cone-bioassay of *An. jeyporiensis* on sprayed walls after one month of DDT indoor residual spraying (1st round 2015) (15 houses, one from each of the 15 randomly selected villages, one house from each village) showed a mortality of 97.8% (n=225). In 2016, one month after 1st round of spraying, the mosquito mortality was 100% (n=215).

Vector abundance indoors (human dwellings and cattle sheds) & outdoors: During Phase 3, the per man-hour density of *An. culicifacies* and *An. fluviatilis* indoors (human dwellings and cattle sheds) (PMDI) and outdoors (PMDO) recorded in the three arms is given in Table 1.6. Consequent to IRS, the density of *An. culicifacies* and *An. fluviatilis* was very low in human dwellings in both Arm 1 and Arm 3 compared to cattle sheds, which were not covered under IRS programme. The density of *An. culicifacies* in human dwellings did

Figure 1.15 Community meeting on indoor residual spraying Format

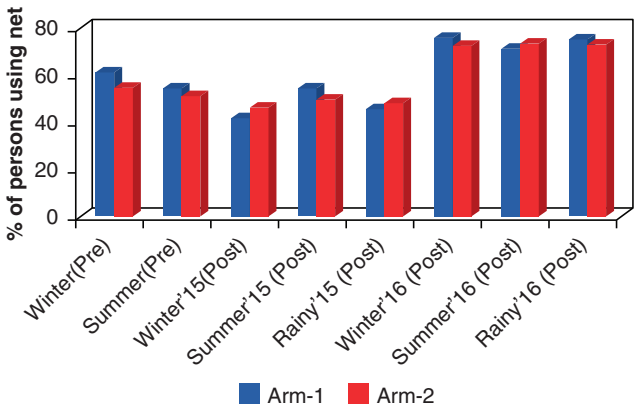


Figure 1.16 LLIN use rate in Arm 1 & Arm 2 during Phase 1 (Pre) and Phase 2 & 3 (post)

TABLE 1.6

Resting density of *An. culicifacies* and *An. fluviatilis* indoors (human dwellings and cattle sheds) and outdoors recorded in the three arms

Arms	Resting density (Number per man-hour)					
	Human dwelling		Cattle sheds		Outdoors	
	Range	Average ± SD	Range	Average ± SD	Range	Average ± SD
An. Culicifacies						
Arm 1 (LLIN + IRS)	0.0 to 0.2	0.0 ± 0.1[a]	0.5 to 22.9	11.2 ± 6.5[b]	0.0 to 0.1	0.0 ± 0.1[e]
Arm 2 (only LLIN)	0.0 to 0.1	0.0 ± 0.0[a]	0.0 to 11.6	4.2 ± 3.1[c]	0.0 to 0.1	0.0 ± 0.0[e]
Arm 3 (only IRS)	0.0 to 0.5	0.1 ± 0.1[a]	1.1 to 21.3	9.1 ± 5.6[d]	0.0 to 0.5	0.1 ± 0.1[f]
An. Fluviatilis						
Arm 1 (LLIN + IRS)	0.0 to 0.0	0.0 ± 0.0[g]	0 to 3.5	0.9 ± 1.1[h]	0.0 to 1.3	0.3 ± 0.3[i]
Arm 2 (only LLIN)	0.0 to 0.1	0.01 ± 0.03[g]	0 to 1.4	0.5 ± 0.5[h]	0.0 to 0.5	0.1 ± 0.1[j]

The average values, column-wise, sharing similar letter do not differ significantly (p<0.05)

not differ significantly between the three arms (F=2.254, p=0.113) whereas in cattle sheds and outdoors it differed significantly (p<0.05). The density of this species (based on cattle shed collections) was lower during December-January, cold months. In contrast, the density of *An. fluviatilis* was higher during November to January. In both human dwellings and cattle sheds, the PMDI of *An. fluviatilis* was not significantly different between the three Arms (P>0.05). However, the PMDO of this species differed significantly between the Arms (p<0.05), significantly higher in LLIN+IRS arm (Arm 1) than the other two Arms (P<0.05).

Parous rate: In Arm 1, the parous rate of *An. culicifacies* was 48.7% (n=353) in winter, 50.1% (n=738) in summer and 47.9% (n=374) in rainy season. The corresponding values for Arm 2 and Arm 3 were 43.1% (n=72), 42.5% (n=318) and 49.4% (n=166), and 44.4% (n=315), 41.0% (n=537) and 47.8% (n=226). The parous rate of *An. fluviatilis* in Arm 1 was 47.9% (n=217) in winter, 37.5% (n=40) in summer and 22.7% (n=22) in rainy season. In Arm 2 and Arm 3, the corresponding values were 38.9% (n=90), 26.1% (n=23) and 37.5% (n=8), and 36.1% (n=97), 0% (n=10) and 26.3% (n=19). In all the three seasons, there was no significant difference in parous rate of the two vector species between the three arms, except summer during which the parous rate of *An. culicifacies* was significantly lower in LLIN and IRS arms than LLIN+IRS arm (p<0.05 by χ^2 test).

Human blood index (HBI): The HBI of An. culicifacies was 0.007 (n=427), 0 (n=174) and 0.007 (n=289) in Arm 1, Arm 2 and Arm 3, respectively indicating a very low anthropophagic behavior of this species. Similarly, a very low anthropophagy was observed in An. fluviatilis, as its HBI was, respectively, 0.006 (n=155), 0 (n=58) and 0.01 (n=76) in the three arms.

Malaria parasite species and incidence: *Plasmodium falciparum* was the predominant species (94%) and around 6% was due to *P. vivax* infection. During the Phase 1, the monthly parasite incidence (MPI) varied from 0.0 to 4.4 (average ± SD = 0.9 ± 1.2), 0.0 to 3.9 (average ± SD= 1.0 ± 1.3) and 0.0 to 3.3 (average ± SD = 0.7 ± 1.0) in Arm 1, Arm 2 and Arm 3, respectively. During the Phase 2 & 3, the MPI varied from 0.0 to 5.5 (average = 0.9 ± 1.3), 0.0 to 5.1 (average = 0.9 ± 1.3) and 0.0 to 2.0 (average = 0.4 ± 0.7), respectively in the three Arms. During Phase 3, among the three Arms, the MPI was lower in Arm 3 (only IRS) (Figure 1.17). However, the MPI did not differ significantly between the three arms (P>0.05).

The study was completed by November 2016.

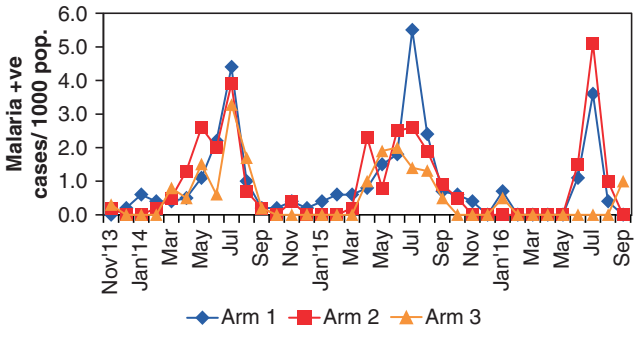


Figure 1.17 Monthly parasite incidence in three arms (from surveillance data collected by VCRC)

1.3.2. 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 and Jambulingam P
Collaborating Institutes: Zoological Survey of India, Chennai; Centre for DNA Finger printing and Diagnostics, Hyderabad and Rajendra Memorial Research Institute, Patna

The kala azar elimination programme in our country aims to achieve the goal by end of the year 2017. While significant reduction has been achieved in the incidence of kala azar cases in the endemic areas, sporadic cases of kala azar are being reported from other states such as Himachal Pradesh, Jammu and Kashmir and Kerala. To sustain the elimination activity, knowledge gap on potential transmission foci and distribution of *Phlebotomus argentipes*, the vector of kala azar is essential. This study is undertaken to describe the reported variants among *P. argentipes s. l.* population in kala azar endemic and non-endemic areas using both morphometric and molecular features. The rationale of the study was already given in the previous annual report. The progress made during reporting year i.e., December 2015–16 is presented here.

Objectives:

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

Various ecotopes namely, delta/ valley, mountain, forest and coastal system, which include, kala azar endemic, non-endemic and areas reporting sporadic cases of kala azar and cutaneous leishmaniasis were chosen to carry out cross sectional surveys. Abundance and distribution of sandflies, including *Phlebotomus argentipes s. l.*, the vector of kala azar and its variants are investigated using both morphometric and molecular tools to target the variant involved in transmission of disease. *P. argentipes s.l.* collected are dissected under a stereo binocular dissection microscope, using sterile dissection needle. The head and genitalia are mounted on clean slides, identified to species. Morphometric features are measured using micrometer while the remaining parts i.e., thorax and abdomen are preserved in eppendorf vial to detect *Leishmania* infection, blood meal source and species/ variant, using molecular markers at the Centre for DNA Finger printing and Diagnostics, Hyderabad. To maximize the identification of relevant polymorphic SNPs, sandfly samples are used for genomic DNA preparation and Hiseq sequencing and genotyping. After discovering relevant SNPs population genetic analysis will be carried out.

During the reporting period cross sectional surveys were conducted by VCRC in 9 areas (Figure 1.18). A total of 2,027 sandfly specimens comprising of 17 species namely, *Phlebotomus argentipes* (47.56%), *P. papatasi* (0.79%), *P. colabaensis* (0.69%), *P. stantoni* (0.05%), *Sergentomyia babu* (30.64%), *S. bagdhadis* (6.91%), *S. baily* (1.53%), *S. chirstophersi* (0.15%), *S. dhandai* (0.39%), *S. indica* (0.35%), *S. insularis* (0.10%), *S. kauli* (1.87%), *S. malabarica* (0.39%), *S. punjabensis* (7.70%), *S. rectangulata* (0.10%), S. shorttii (0.25%) and *S. zeylanica* (0.54%) was recorded during the collection.

P. argentipes was obtained from all the study areas, except in Jammu and Kashmir region, where only a male specimen was obtained from Vijayapur in Kathuva dt.

The morphometric features namely, head width, interocular distance, length of each segment of the palp (1–5), length of antenna segment 3–5, i.e., A3, A4, and A5, length of ascoid i.e., *sensilla chaetica* in A3, length and width of pharynx, length and width of wings in males and females; length of spermatheca,

Figure 1.18 Map showing sandfiles collection sites

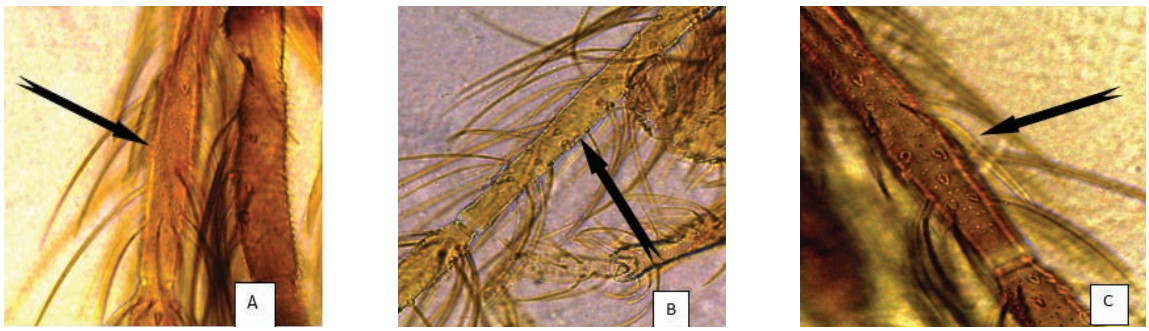


Figure 1.19 Antenna 3 with ascoid in three variant of *P. argentipes* recorded in kala azar endemic areas of Bihar (A), cutaneous leishmaniasis cases recorded areas of Melaamala (B) and non-endemic areas of Pondicherry (C)

length of individual duct of each spermatheca, length of circus in females; and length of genital pump, genital filament, coxite, style and terminal spine in male specimens of *P. argentipes* were measured using micrometer, fitted with an Olympus binocular microscope (Model: CHS, Olympus Optical Co Ltd, made in Japan). All measurements recorded were converted to µm. Mean values of the morphometric characters indicate occurrence of three sibling species within *P. argentipes* s. l. population. The variation in the length of *sensilla chaeticum* in antenna 3 of *P. argentipes* s. l. collected from different study areas (n=10 in each area, except Kathuva where only a male specimen was processed) is depicted in Figure 1.19 (A, B and C). Identification of species complex using molecular markers and mapping their distribution are on progress.

1.3.3. Prevalence and distribution of haemoglobinopathies (Sickle cell and Thalassemia) and G6PD deficiency in relation to malaria among tribal groups in Odisha State, India

EM 1607: Jan 2016 – Dec 2017

Gunasekaran K, Sahu SS, Sankari T, Krishnamoorthy N, Vector Control Research Centre, Puducherry , Behera KP, Padhi A, CDMO Office (State Health Department), Koraput
Collaborating Institutes/Departments: State Health Department, Govt. of Odisha

The State of Odisha with a population of 40 million has 22% tribal population belonging to 62 ethnic groups. Malaria has been a major public health problem among tribes who harbour malaria parasites including *Plasmodium vivax* for decades. Primaquine (adult dose: 15 mg per day for 14 days) is the drug being administered to patients suffering from vivax malaria. However, adequate data on G6PD status among the tribal people are lacking. Therefore, this study will help in better management of *P. vivax* cases.

Objective:

- To assess the extent of G6PD deficiency in relation to treatment of *vivax* malaria among different tribes.

Based on the occurrence of incidence of *P. vivax* malaria and presence of different tribes, six Blocks, four from Koraput and two from Malkangiri district have been selected for the study. From each Block, five villages were randomly selected for assessing the prevalence of G6PD deficiency. The required sample size to represent each Block/tribe with 95% confidence was decided assuming an average disorder prevalence of 10% and allowing a precision of 4% (Table 1.7). The sample size for each village was estimated based on proportion to the population. Clearance has been obtained from the Centre's Human Ethics Committee for collecting blood samples from the patients attending OPD of the Dt. Hq. Hospital, Koraput/respective CHCs and from the people at their villages. Informed consent was obtained from each person tested.

The survey has been initiated in Koraput district since July 2016. Both hospital/CHC and community based surveys were carried out. A total of 476 persons were screened at the district headquarters hospital, Koraput, Community Health Centre (CHC), Borigumma and Narayanpatna and villages of these two CHCs and the blood samples were tested using qualitative method, dichloro phenol indo-phenol dye reduction test. Out of them, 13 persons (2.7%) were found to have G6PD deficiency. They will be confirmed for the deficiency by quantitative G6PD assay. All the 476 people were also screened for malaria parasites and out of the 369 blood smears examined 25 were malaria positives; two for *P vivax* and the remaining for *P. falciparum* (Table 1.8). The *P. vivax* positive persons were not found with G6PD deficiency.

TABLE 1.7

Study sites with tribes and the sample size proposed in each Block for screening

District	Block	Population	Tribe	Tribal Population	Malaria +ves*	*Pv* cases*	Sample Size
Koraput	Narayanpatna	45,280	Kondha	35,292	4006	171	215
	Borigumma	1,55,095	Porja	77,913	664	31	216
	Lamtaput	59,374	Godva	27,811	3188	179	215
	Kundra	72,700	Bhumia	36,745	1410	92	215
Malkangiri	Korukonda	1,16,748	Koya	71,055	2881	57	216
	Khairput	46,556	Bonda*	5,600	1142	56	209

** 2013 data, obtained from District Health Department*

TABLE 1.8

Details of samples collected and analyzed for G6PD deficiency in Koraput district

Sl. No.	Place of screening	Number of villages surveyed	Total samples collected	G6PD deficient	Malaria +ves*	*P. v*	*P. f*
1	Koraput Dt. Hq. Hospital	–	20	0	2	1	1
2	Borigumma CHC	–	23	2	4	1	3
3	Narayanpatna CHC	–	24	1	7	0	7
4	Borigumma CHC villages	4	217	9	3	0	3
5	Narayanpatna CHC villages	5	192	1	9	0	9

1.3.4. Field evaluation of DawaPlus 3.0 and DawaPlus 4.0 long-lasting insecticidal nets from Tana Netting, UAE against natural populations of *Anopheles culicifacies* s.l. and or *Anopheles fluviatilis* s.l. in experimental huts in Odisha, east-central India

EM 1602: May 2016 – Apr 2017

Gunasekaran K, Sahu SS, Vijayakumar T, Subramanian S, Jambulingam P
Collaborating Institutes/Departments: WHOPES, WHO, Geneva & Walloon Agricultural Centre (CRA-W), Pesticide Research Dept., Rue de Bordia, Belgium

In view of the operational constraint such as low retreatment rates of bed nets, long-lasting insecticidal nets (LNs), which require no further treatment throughout their expected life span of about three years or even more, have been developed for use. The WHO Pesticide Evaluation Scheme (WHOPES) has already granted full / interim recommendations to some of the LNs and a few more are under initial phases of evaluation. DawaPlus 3.0 & DawaPlus 4.0 are a new generation LNs with the pyrethroid insecticide, deltamethrin and the oxidase synergist piperonyl butoxide (PBO). Combination or Mixture LNs such as these with PBO may have application against resistant mosquitoes, particularly those whose resistance is based on oxidative metabolism. DawaPlus 3.0 and DawaPlus 4.0 are now made available by WHOPES for Phase II evaluation.

Objectives:

- The overall objective was to determine and compare the efficacy of DawaPlus 3.0 and DawaPlus 4.0 against a positive control deltamethrin Ln (DawaPlus 2.0) and an untreated negative control polyester net.

Specific objectives:

- Determination of the efficacy in terms of mortality and blood-feeding inhibition of unwashed and 20 times washed DawaPlus 3.0 and DawaPlus 4.0.
- Determination of deterrence, induced exiting, personal protection and mass killing effect of unwashed and 20 times washed DawaPlus 3.0 and DawaPlus 4.0.
- Chemical analysis of unwashed and 20 times washed DawaPlus 3.0 and DawaPlus 4.0 to determine content of deltamethrin and PBO.
- Recording of the perceived side-effects of the DawaPlus 3.0 and DawaPlus 4.0 among the volunteers sleeping under the LNs in the experiment huts.

The candidate LN, DawaPlus 3.0 from Tana Netting, UAE, is a combination LLIN comprised of side panels made of knitted poly-filament polyester fibres (42 g/m²) coated with 2.5 g ai/kg deltamethrin (105 mg/m2), and roof made of polyethylene (40 g/m²) incorporating 3 g ai/kg deltamethrin (120 mg ai/m2) and 12 g/kg PBO (480 mg/m2). The second candidate LN, DawaPlus 4.0 also from Tana Netting, is a mixture net comprised of side and roof panels made of polyethylene (40 g/m²) incorporating 3 g ai/kg deltamethrin (120 mg ai/m2) and 12 g/kg PBO (480 mg/m2).

DawaPlus 3.0 and 4.0 were compared against DawaPlus 2.0, the WHOPES recommended deltamethrin LN (positive control) and untreated net (negative control). The efficacy was evaluated in experimental huts, which stimulate domestic houses, against a wild, free flying deltamethrin resistant *Anopheles culicifacies* sensu lato in terms of deterrence, induced exiting, blood-feeding inhibition, mortality, personal protection and mass killing effect in Malkangiri, Odisha State following the WHO guidelines for laboratory and field testing of LNs.

The evaluation of included nets of seven arms viz., 1) Unwashed DawaPlus 3.0, 2) DawaPlus 3.0 washed 20 times, 3) Unwashed DawaPlus 4.0, 4) DawaPlus 4.0 washed 20 times, 5) Unwashed DawaPlus 2.0 (positive control), 6) DawaPlus 2.0 washed 20 times (positive control) and 7) Untreated polyester net (negative control). There were six replicate nets in each arm, used in six experimental huts on rotation. Each arm had also three additional nets for conducting bioassay and chemical analysis prior to any wash, after 10 washes and after 20 washes.

Net washing: The candidate LLINs (DawaPlus 3.0 & 4.0) and nets of the other arms were received from the WHOPES. All nets were coded to indicate the arm and the replicate nets of each arm.

Nets of Arm 2, 4 and 6 were washed 10 times and continued up to 20 times. After washing, the nets were shifted to the field site at Malkangiri for hut evaluation. Ethical clearance to engage human volunteers (14 persons) for sleeping under test nets in experimental huts was obtained from the Human Ethics Committee of VCRC. A contingency insurance policy with an individual insured has been taken for the volunteers.

Suitability of the experimental huts, in terms of yielding comparable indoor resting density with that of village huts, tightness with 80% recovery of released mosquitoes and absence of scavengers, was ensured prior to hut evaluation.

Hut evaluation: Over 42 collections conducted during a period of 7 weeks, the mean density of *An. culicifacies* entered the experimental huts with arms 1, 2, 3, 4, 5, 6 & 7 was 0.24, 0.45, 0.29, 0.5, 0.40, 0.73 and 6.28, respectively. The exit rate was the highest in in Arm 1 (70.0%) and lowest in Arm 7 (23.9%). Feeding rate was 0.0%, 42.1%, 66.7%, 66.7%, 76.5%, 67.5% and 93.9 % in arms 1 to 7, respectively. The feeding rate was higher in Arm 7 (93.9 %) and zero in Arm1. The immediate, delayed and total mortality rates of the vector species is given in Table 1.9.

Perceived side effects and benefits: Interview of the 14 volunteers revealed that no volunteer suffered either from nose irritation nor itching of body parts like face and hands etc. All volunteers who slept under different arm nets stated that they did not get any odour from the nets. They had the perceived benefit of reduced mosquito bites in the huts and an undisturbed night sleep throughout the study period.

Cone-bioassays: The cone-bioassay conducted prior to any wash and after 10 and 20 washes 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%. The bioassay results with *An. culicifacies* prior to hut evaluation showed a higher mortality in Arm 1 & Arm 3 and in Arm 7 there was no mortality (Table 1.10).

TABLE 1.9

Mortality of *An. culicifacies* in treated and untreated arms

Arms	No. of collections	Number collected	Number dead	Immediate Mortality	Delayed Mortality (%)	Total Mortality (%)
Arm 1	42	10	5	0.0	50.0	50.0
Arm 2	42	19	6	0.0	31.6	31.6
Arm 3	42	12	5	0.0	41.7	41.7
Arm 4	42	21	7	0.0	33.3	33.3
Arm 5	42	17	3	0.0	17.6	17.6
Arm 6	42	31	8	0.0	25.8	25.8
Arm 7	42	264	0	0.0	0.0	0.0

Personal protection and mass killing effect: The percent personal protection was 100.0, 88.6, 88.6, 86.4, 86.7 and 83.7 with Arm 1 to 6, respectively and the percent mass killing effect of Arms 1 to 6 was 1.9, 2.3,1.9, 2.7,1.1, and 3.0.

Cone bioassay of nets after the hut evaluation and chemical analysis of nets of all the seven Arms for insecticide content prior to any wash, after 10 and 20 washes and after hut evaluation need to be carried out.

TABLE 1.10

Results of cone-bioassays

Arms	Prior to any wash*		After 10 washes*		After 20 washes*		Prior to hut evaluation#	
	No. exposed	Corrected mortality (%)	No. exposed	Corrected mortality (%)	No. exposed	Corrected mortality (%)	No. exposed	Corrected mortality (%)
X 1	50	100	50	100	50	100	50	48
X 2	50	100	50	100	50	100	50	34
X 3	50	100	50	100	50	100	50	48
X 4	50	100	50	100	50	100	50	4
X 5	50	100	50	100	50	100	50	32
X 6	50	100	50	100	50	100	50	26
X 7	50	0	50	0	50	0	50	0

**Susceptible An. stephensi was used for cone-bioassays prior to and after washes*
 # Resistant An. culicifacies was used for cone-bioassay prior to hut evaluation

1.4 DENGUE / JE / KFD

1.4.1. Studies on Epidemiology of Dengue in forest fringe areas of Kerala

IM 1303: Sep 2013 – Aug 2016

N. Pradeep Kumar, K.N. Vijaya Kumar, Ambili Kumar, K.S. Snehalatha, Abidha, Krishnamoorthy K & Jambulingam P

The project studies were initiated during 2013 and baseline information on the risk factors involved in the transmission of Dengue in Kanjirappally taluk were delineated.

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 IVM strategy to prevent/contain of dengue outbreaks in these ecosystems.

The study areas were Kanjirappally town (urban) and Erumeli and Koruthodu (forest fringe, rural).Twenty two species of mosquitoes were recorded (n=12421) and the most predominant species was *Stegomyi albopicta* (39.46%). In the urban area, while both the species *(St. aegypti* and *St. albopicta)* were prevalent, only *St. albopicta* was recorded in the forest fringe region and the peak was recorded during the pre-monsoon summer season. For *St. aegypti* this could be attributed to the acute water shortage in the urban area, leading to containers water storage habitats, which contributed to 83.38% of the species population. *St. albopicta* had diverse breeding sources which proliferated due to intermittent rainfall during the summer season.

Infection status in the vector population: Natural infection with DENV infection was recorded only in *St. aegypti*. A total of 463 specimens of *St. aegypti* (266 females & 197 males) collected in adult/immature collections were processed from urban area, in 234 pools. Of this two semi-gravid pools (June & July 2014) from urban area (indoor resting collection) were found positive for DENV infection. One pool had DENV4 and another had DENV2 infection. Processing of 371 pools of *St. albopicta* with 1035 specimens from urban and rural areas (642 females & 393 males), did not show any positivity for DENV infection.

Blood meal source of mosquitoes: DNA barcode analysis was done to determine the source of mosquito blood meal and vector host association. The COI sequences of 46 specimens (*St. aegypti* = 20; *St. albopicta* = 26) of vertebrate host-species of blood meal were amplified and custom sequenced and all were found to be from *Homo sapiens*.

Incidence of Dengue: Hospital based surveys revealed a reduction in the incidence of disease (per 100,000 population) from 231.60 to 112.28 during these years. IDSP reports were less due to non-reporting of cases attending private medical institutions.

Serological surveys: Sample blood survey was carried out in the study villages, both rural and urban, to assess the seroprevalence of DENV in human population. The sample size was determined based on a pretest survey. A systematic sampling technique, with a random start, taking household as sampling unit was performed for the study. Male to female ratio in the study area was 1.02. The number of samples collected from urban and rural areas was 473 and 468 respectively. Out of 941 samples collected, 493 (52.39%) were found to be serologically positive for Dengue IgG. The seroprevalence for the study areas, viz., urban and rural was found to be 60.46% and 44.23% respectively. Seroprevalence was significantly higher in the urban areas (60.46%) compared to rural (44.23%) settings (X^2= 25.316; p<0.0001). There was no significant difference in seroprevalence rates between males and females (p=0.345). Seroprevalence was significantly higher in the age group 40-50 and 50-60 years (p<0.01).

Recommended IVM strategy: Forest fringe areas were worst affected region during 2010 outbreak of Chikungunya/Dengue fevers in Kottayam District of Kerala. Efforts for vector control by the state Health department reduced the vector population resulting in a significant reduction of cases reported in this region. We did not notice

any sylvatic strain of DENV in the region, as vector population survey for a period of 2 years in these villages did not yield any infected specimens. Also, none of the DENV serotypes recorded in the urban area had a significant genetic similarity with the reported sylvatic strains of DENV. 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 comparatively higher than the villages. Community education campaign and introduction of small larvivorous fishes involving community during the peak summer season would prevent dengue outbreaks in the area.

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

IM 1304: Jan 2013 – Dec 2016

Krishnamoorthy K and Nandha B
Collaborators: NVBDCP, Pondicherry

Incidence of *Aedes* borne dengue and chikungunya is reported from different parts of Pondicherry. As many as 2215 and 1322 cases and 1 death were reported in 2013 and 2014 respectively. Though new cases were in the declining trend, every year cases were reported. In the absence of any organised vector management, cases continue to occur and the problem has become more acute in rural areas. This study aims at developing and demonstrating integrated vector management (IVM) focusing on empowering communities for the prevention and control of dengue.

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

Two PHCs viz., Mannadipet and Ramanathapuram with population of 15331 (9 villages) were selected as intervention arm and one PHC (Thirukanur) with a population of 10528 in five villages as control arm. Dengue cases are reported every year in these PHCs. Community Readiness assessment study in these villages also indicated low level community readiness in undertaking preventive measures, demanding a system for constant motivation.

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 of discarded containers/utensils, discouraging the practice of discarding utensils/containers (rain dependent) and their clearance as well as managing the water storage containers (cisterns and plastic drums) are the key messages besides demonstrating source reduction during the house visits are the task entrusted with the volunteers. The results of the study 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 2016. A total of 600 households were visited by students during 2016 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 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 in middle and high schools were trained on assessing the breeding sources in and around their houses. A simple pictorial assisted proforma in local language was prepared. About 75% of the students could complete the forms and the leaders in the class were given preliminary training on the distribution, collection and verification of forms for its completeness. Fortnightly surveys were conducted. A total of 1793 houses were enumerated in the intervention area and found that 13.8% and 4.5% of the houses had discarded and water storage habitats where as it was 25.8% and 5.4% in the control arm in 1753 houses. The percentage of houses with vector breeding in discarded and water storage habitats in the intervention arm was 2.24% and 0.96 % and in the control arm it was 5.2% and 1.2% respectively.

A teacher from each school has taken responsibility to ensure that the activity is continued. As a follow-up activity, a training workshop was conducted at the Centre jointly with State NVBDCP and two students in each of the selected class and a teacher from these study areas were trained.

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 monitor the situation and wherever and whenever interventions are indicated, appropriate action will be carried out by the state NVBDCP.

1.4.3. Monitoring JE vector abundance in an area with mixed vegetation and varied water bodies using RS and GIS

Mar 2015 – Feb 2017

Raju KHK, Rajavel R, Jambulingam P & Sabesan S
Collaborators: National Remote Sensing Centre, Hyderabad

Incidence of JE in Uttar Pradesh (UP) contributed to more than 75% of the total cases in India. In UP, seven of the total of 71 districts, are highly endemic, and among them, Gorakhpur is worst affected. In Gorakhpur, the VCRC has carried out a research cum intervention study during the years 2013–15. For monitoring the vector abundance in Gorakhpur, the use of remote sensing (RS) and GIS has been explored with the available data on vector density, retrospectively.

Objectives:

- Development of model calibration for land use / land cover in Gorakhpur
- Ascertain the relationship between the JE vector abundance and ground characteristics through RS indicators

Study sites: In Gorakhpur, two JE endemic Blocks viz., Campierganj and Belghat each with five villages were selected. There is mixed vegetation, though paddy cultivation is predominant with large number of varied water bodies. Paddy is grown in two seasons ('rabi': Nov–Mar & 'kharif': Jun–Oct). However, JE is reported mainly in 'kharif' season, and hence we focused our attention during this season.

Development of model calibration: For monitoring the vector abundance, the use of RISAT 1 data was demonstrated successfully in areas where the rice alone (monoculture) is grown (Bellari district, Karnataka) in an earlier study. In the present study, the availability of RISAT 1 data was searched for Gorakhpur in the designated website of NRSC (ISRO), Hyderabad. The data was available only partially (a part of the study area, for two time points) and further it was learnt that the RISAT 1 data will be made available to the users only on request, with firm order in advance. As it was not possible to obtain the RISAT 1 data on old dates to determine the association, if any with the available vector data, retrospectively, it became necessary to look for an alternate satellite data

which could be used for areas with mixed vegetation and varied water bodies. For this purpose, Moderate Resolution Imaging Spectroradiometer (MODIS) data was preferred, as it provides substantially improved radiometric and geometric property of mixed vegetation types in an area within a season / annual cycle. Further, it is capable of identifying phenologic behavior characterized by mixed vegetation growth and senescence periods, which is common in crop land regions (similar to that of our study areas). MODIS data is available on public domain, at fortnightly interval, with 250 m resolution. By registering as one of the user scientists, the MODIS data was downloaded authentically, and constructed the paddy crop time- based phenology profiles for two years i.e., 2014–2015, focusing the land use / land cover (LULC) during 'kharif' season. Month wise normalized difference vegetation index (NDVI) values were derived from MODIS imageries following the standardized protocol.

Association between the ground characteristics (LULC) and vector abundance: The derived NDVI values during 'kharif' season ranged between 0. 3 (May/Jun) – 0.7 (Aug). From vegetative stage of paddy during July, the value (0.5) started increasing, and reached a high in its flowering stage during Aug (0.7). Then, the values decline in subsequent stages of paddy, despite other vegetations remains without much change in canopy. The vector data (per man-hour density of *Culex tritaeniorhynchus*) generated during the above period under another project was used for the current study to verify the relationship with LULC (mixed vegetations, and water bodies), using the derived NDVI values. Regression analysis revealed a significant association (R^2 = 0.7252) between NDVI and vector density (Figure 1.20).

The NDVI values derived from MODIS imageries could be used as 'proxy' for monitoring the vector abundance in mixed vegetation areas (like Gorakhpur) where paddy cultivation is predominant.

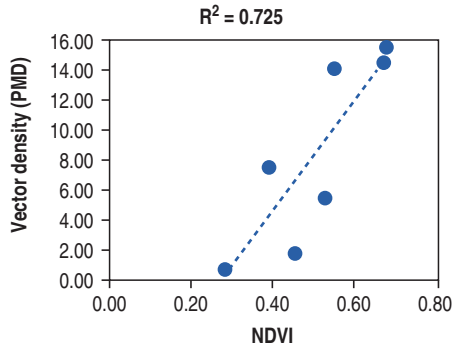


Figure 1.20 Association between NDVI and Vector density

1.4.4. Entomological investigation on the suspected outbreak of Japanese encephalitis in tribal villages of Malkangiri district, Odisha State

Aug 2015 – Jul 2016

Sahu SS, Gunasekaran K, Dash S, Rao SP, Sonia T, Muthukumaravel S, Pandit R, Pradhan MM, Jambulingam P
Collaborating Institutes/Departments: NVBDCP, Bhubaneswar, Govt. of Odisha

Malkangiri, the southernmost district of Odisha State, recorded 38 deaths of children due to suspected JE during the last three years. In 2014, eight deaths of children were reported in seven villages of Korkunda and Kalimela CHCs of the district. To implement appropriate measures to prevent the disease outbreak, the local government requested VCRC to undertake a study to assess the entomological risk factors for transmission of JE. The proposed study in the suspected JE reported villages assessed the mosquito species composition, relative and seasonal abundance of mosquitoes with particular reference to the known JE vector species and screened mosquito species for JE virus infectionfor one year, covering the two favourable seasons of JE transmission i.e. rainy and post rainy.

Objectives:

- To monitor abundance (density), human blood index, survival and dusk index of known JE vectors
- To screen mosquito species for JE virus infection

Progress:

Immature survey: The density of culicine immature was 0.14 per dip in pits and paddy fields and 0.08 per dip in ponds. The emerged mosquitoes included *Cx. tritaeniorhynchus* and *Cx. vishnui*, the known JE vectors.

Adult mosquito collections: A total of 3,085 adult mosquitoes, belonging to 24 species of two genera (*Culex* and *Anopheles*), were obtained from the adult collections and that included eight *Culex* and 16 *Anopheles* species. Among the total (n=3,085) mosquitoes collected, 2,347 belonged to the nine (7 *Culex* and 2 *Anopheles* species) recognized JE vectors and among them *Cx. vishnui* was the predominant species (38.3%). The proportion of the other species is shown in Figure 1.21.

Month-wise per man-hour density (PMD) and dusk index (DI) of the two primary JE vector species, *Cx. tritaeniorhynchus* and *Cx. vishnui*, are

shown in Figures 1.22 & 1.23. Both PMD and DI showed a similar seasonality, higher during July to November, monsoon months and lower from December to June, winter and summer months. The higher density of the vector species during rainy season coincides with the period of paddy cultivation.

Blood meal analysis and detection of JE virus: A total of 468 blood meals of the nine known JE vector species were tested for the source of feeding; 98.1% was found to be bovine source and 1.9% fed on human, suggesting a very low anthropophagic behavior of the vector species. Specifically, while 2.6% (n=153) of *Cx. vishnui* blood meals was tested positive for human, none of the 59 blood meals of *Cx. tritaeniorhynchus* reacted with human antiserum. In total, 1,836 mosquitoes of the known vector specie were RT-PCR assayed for detection of JE virus and none was found positive with JE virus.

Summary & conclusion: In the study area, extensive rice cultivation favours breeding of the recognized JE vector species. Though, overall, dusk index of the vector species was low, among the three seasons, it was relatively higher during rainy season. Also, a higher per man-hour vector density was recorded during rainy/rice cultivation season during which outbreaks of suspected JE/AES and deaths occurred. The vector species exhibited a poor anthropophagy (low HBI) and RT-PCR assay did not show any infection with JE virus. However, the investigation done during the recent (September–October 2016) outbreak of suspected JE/AES in the same area detected a positive pool (25 mosquitoes) of *Cx. vishnui* for JE virus (n= 58 pools). With all these facts, it could be concluded that the area was receptive with entomological risk factors for JE transmission, but might be at low level.

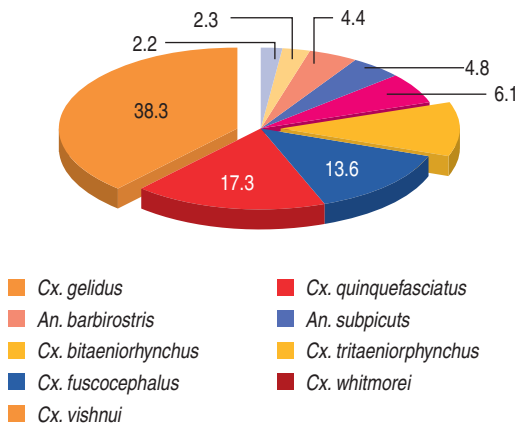


Figure 1.21 Proportion of the recognized JE vector species from adult collections

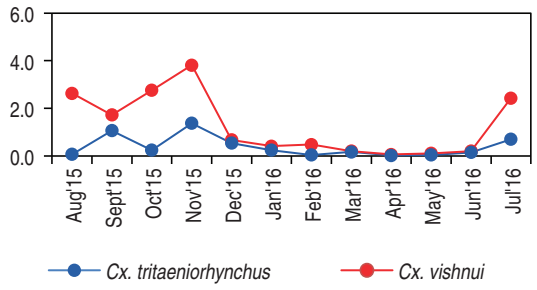


Figure 1.22 Per man-hour density of *Cx. tritaeniorhynchus* and *Cx. vishnui* during the study period

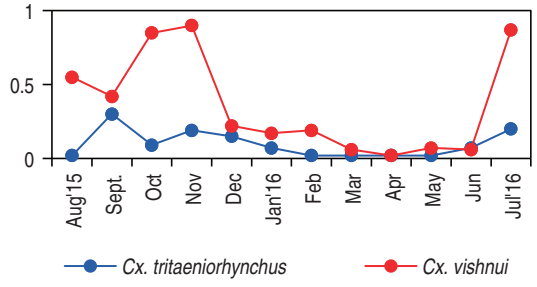


Figure 1.23 Dusk index *Cx. tritaeniorhynchus* and *Cx. vishnui* during the study period

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

EM 1504: Aug 2015 – Jul 2017

Pradeep Kumar N, Srinivasan R, Natarajan R
Collaborating Institutions: National Institute of Malaria Research

Aedes aegypti and *Ae. albopictus* are the two species among the mosquito vectors that are of prime concern at present due to the frequent outbreaks of Dengue and Chikungunya in the many parts of the country and the resulting morbidity and mortality. While *Ae. aegytpi* is known to be a peri-domestic species, the recent reports of co-habitation of *Ae. albopictus* with this species in peri-domestic habitats and the purported replacement of *Ae. aegypti* by *Ae. albopictus* in several areas results in a paradigm that needs to be studied. Besides this, the *albopictus* subgroup consists of 9 species, out of which 4 are known to occur in India, namely *Ae. albopictus*, *Ae. novalbopictus*, *Ae. pseudoalbopictus* and *Ae. subalbopictus*. Although information on the distribution and ecology of *Ae. albopictus*

is available, such information for other species of the subgroup is scanty. The proposed project aims to generate information on these aspects besides screening the population of these species for Dengue and Chikungunya viruses. It would serve as the baseline data for developing and implementing suitable surveillance mechanisms for the vectors of dengue and chikungunya.

Objectives:

- To study the ecology, distribution and seasonal prevalence of the species of *albopictus* subgroup and *Ae. aegypti* in different ecosystems.
- To define the breeding habitats and study the association of species.
- To study the host preference of the species *Ae. albopictus* subgroup and *Ae. aegypti* and screen the different species for dengue and chikungunya virus infection.

The study area: The study is carried out in five districts in Kerala namely, Wayanad, Ernakulam, Pathanamthitta, Idukki and Thiruvananthapuram. The areas were selected based on geographic distribution of specific ecosystems in each district, which favours abundance and proliferation of *albopictus* subgroup besides, the incidence of *Aedes* transmitted diseases namely, Dengue and Chikungunya over the past few years (Figure 1.24). Wayanad district situated in the north east encompasses the Western Ghats with dense forest and deep valleys and has plantations of coffee, tea and rubber. Ernakulam is a coastal district. Pathananmthitta district is with extensive rubber, pineapple plantations and its hills are blanketed with thick forests. Idukki district is known for its mountainous peak and dense forest, covers almost 50% of the district besides, several plantation areas of mainly tea. Thiruvananthapuram district not only has a long coastal area but also the high hill ranges of the Western Ghats which includes wild life sanctuaries with wide variety of vegetation, ranging from tropical wet evergreen forests to grass lands. Thiruvananthapuram city, considered as a metropolis provides a typical urban situation.

Extensive surveys were carried out during pre-monsoon in two districts, namely Idukki and Patrhanamthetta and in all the five districts during monsoon season. Mosquito collections were made in 36 villages, selected based on the earlier report on dengue/ chikungunya cases within the districts (Figure 1.24). Both immature and adult mosquitoes were collected as per standard procedure. Resting collections were made indoors. Immatures of *Aedes* spp. were collected in

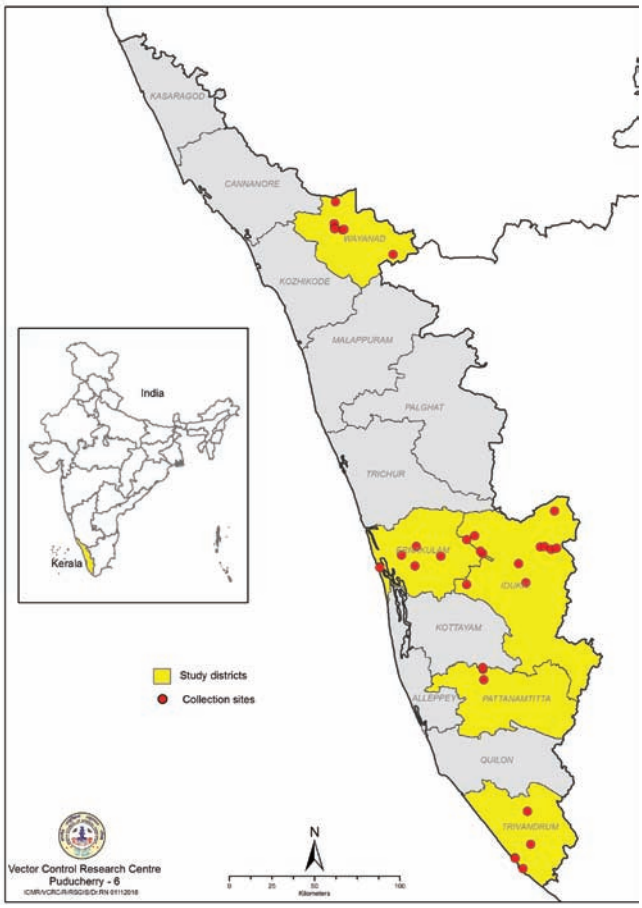


Figure 1.24 Map showing the study districts and collection sites

varieties of habitats. Both larvae and pupae were reared to adults in the field laboratory. Adult mosquitoes obtained in the resting collections and those emerged from larvae/ pupae from immature collections were identified to species. After identification, *Aedes aegypti*, *Aedes albopictus* and *Aedes* subgroup samples were preserved as per molecular protocol and given for virus isolation and DNA barcoding.

A total of 41 species of mosquitoes was recorded in this study that include *Ae. aegypti* and *Albopictus* subspecies namely, *Ae. albopictus, Ae. pseudalbopictus, Ae. subalbopictus* and *Ae. novalbopictus*. While *Ae. albopictus* was recorded in all study districts, *Ae. novalbopictus* and *Ae. subalbopictus* were collected from Wayanad district. *Ae. pseudo albopictus* spotted from in Ernakulum district. *Aedes aegypti* was recorded in Ernakulam and Wayanad districts. *Ae. albopictus* was found to breed in a variety of habitats both in domestic (defrost tray) and peri domestic containers and found in association with *Ae. aegypti* and *Ae. novalbopictus* in many habitats indicating its adaptability and ascendancy when compared with other mosquito species. Screening of these species for dengue and CHIKV is on progress.

1.4.6. 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 2017

Jambulingam P, Rajavel AR, Subramanian S, Gunasekaran K
Collaborating Institutes/Departments: CRME Madurai, NIV Pune & State Health Dept., Govt. of UP

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

Baseline data: The study was initiated in August 2013 and since then collection of baseline data on entomological parameters was continued through 2014 and 2015 in Campierganj and Belghat (intervention blocks) in Gorakhpur District and in Majhgawa (comparison block) in Deoria District (Figure 1.25).

Figure 1.25 Map showing the intervention blocks: 1. Campierganj (LLIN), 2. Bhathat and 3. Belghat (IRS) in Gorakhpur District

Intervention measures: The intervention protocol of the study was to carry out indoor residual spraying (IRS) with lambdacyhalothrin 10% WP at 25mg/m² in two blocks, namely, Belghat and Bhathat and to distribute long lasting insecticidal nets (LLINs) in Campierganj block, keeping Majhgawa block in Deoria district as the control for comparison. Accordingly, IRS was carried out in the two blocks during June-July 2015. The house/room spray coverage details and the impact of spraying on vector density in the intervention block in comparison to the control block was already communicated (Annual Report 2015).

Distribution of LLIN: In Campierganj block, distribution of LLIN was started in August 2015, but it was possible to cover only four sub-centres, distributing 8100 nets, since it had to be suspended due to the logistic problems as a consequence of the gram panchayat elections. It was also realized that family-card-based distribution of LLINs through identified distribution centres was not feasible and will not cover adequately all villages of the block. Two to three alternate strategies of LLIN distribution were further tested in the villages, but none was found to be successful. Due to such practical problems, implementation of LLIN distribution was delayed. It was decided finally to distribute the nets to the households on the basis of the ration cards registered online and with this plan net distribution was resumed in July 2016 in index villages, distributing a total of 3947 nets. In the other villages, distribution was begun in September 2016 and expected to be completed by October/November 2016. So far, 25 villages were covered by distributing 13,395 nets, thus making a total distribution of 25,442 nets in Campierganj block (Table 1.11).

Indoor residual spraying: Following the one round of indoor residual spraying in Belghat and

TABLE 1.11

Distribution of long lasting insecticidal nets (LLINs)

Period	Block	Areas covered	No of LLINs distributed
Aug 2015	Campierganj	4 SCs	8,100
Jul 2016	Campierganj	Index villages (5)	1,760
	Khorabar	Index villages (5)	2,187
Sep 2016	Campierganj	24 villages, Ongoing	13,395
		Total	**25,442**

Bhathat blocks in 2015, IRS was again carried out in June 2016 in these two blocks covering eight (house spray coverage=95.1%, n=9,914; room coverage=80.1%, n=39,117) and nine sub-centres (house spray coverage=91.3%, n=10,978; room coverage=87.8%, n=38,294) respectively, including the index villages. Besides human dwellings, 4,462 cattle sheds and 16 pig sties in Belghat SCs and 1,119 cattle sheds and 16 pig sties in Bhathat SCs were sprayed (Table 1.12 & 1.13). After a gap of three months, i.e. in September 2016, one more round of IRS was done in the index villages of the two blocks (five villages in each block). In the index villages of Belghat, 1,611 houses out of 1,629 (98.9% coverage) and 4,974 rooms out of 6315 (78.8% coverage) and in the Bhathat index villages, 1,675 houses (91.2%, n=1837) and 5,248 rooms (85.2%, n=6,168) were sprayed. In addition, 7,20 cattle sheds and 1 pig sty in Belghat index villages and 373 cattle sheds in Bhathat villages were sprayed (Table 1.14).

TABLE 1.12

First round of indoor residual spraying in 2016 in Belghat block, Gorakhpur district: Sub-Centre-wise house and room coverage (*The Sub-Centre includes one of the index villages)

Sub-Centre	To be Sprayed		Sprayed		Houses Locked/ Refused	Sprayed	
	Houses	Rooms	Houses	Rooms		Cattle Sheds	Pig Sties
Belghat-1	1532	6238	1518	4951	14	658	2
Belghat-2	1422	5215	1350	4279	72	391	1
Rasulpur	1261	5043	1213	3915	48	618	2
Bahadurpur*	942	3563	887	2862	55	502	0
Gayghat*	906	3290	875	2723	30	407	3
Basantpur*	1534	5983	1472	4750	62	793	8
Malav*	1382	6046	1346	4687	38	644	0
Barigaon*	938	3738	771	3145	165	449	0
Total	**9914**	**39117**	**9432**	**31312**	**482**	**4462**	**16**
Coverage	–	–	**95.1**	**80.1**	–	–	–

*Additional adjoining village

TABLE 1.13

First round of indoor residual spraying in 2016 in Bhathat block, Gorakhpur district: Sub-Centre-wise house and room coverage (*The Sub-Centre includes one of the index villages)

Sub-Centre	To be Sprayed		Sprayed		Houses Locked/ Refused	Sprayed	
	Houses	Rooms	Houses	Rooms		Cattle Sheds	Pig Sties
Jungle Dumri-2*	1374	4610	1313	4058	61	153	7
Dumri-1*	1731	5510	1646	4960	85	213	0
Bhathat*	2032	6743	1884	5924	148	117	1
Gulhariya*	1856	6532	1638	5772	218	182	4
Ghoradaur*	1577	5351	1396	4836	181	122	4
Parsauna	214	680	200	597	14	23	0
Amwa	219	776	144	653	75	23	0
Rampur Bujurg	484	1816	446	1683	38	44	0
Bailo	1491	6276	1356	5128	135	242	0
Total	**10978**	**38294**	**10023**	**33611**	**955**	**1119**	**16**
Coverage	–	–	**91.3**	**87.8**	–	–	–

*Additional adjoining village

TABLE 1.14

Second round of indoor residual spraying in 2016: House and room spray coverage in index villages in the two IRS blocks

Sub-Centre	Index villages	To be Sprayed		Sprayed		Houses Locked/ Refused	Sprayed	
		Houses	Rooms	Houses	Rooms		Cattle Sheds	Pig Sties
Belghat block								
Belghat-1	Belghat-1*	324	1226	326	960	0	62	0
Bahadurpur	Bahadurpur	242	890	239	680	3	123	0
Gayghat	Gayghat	156	705	154	564	2	94	1
Basantpur	Bharsi	254	919	252	732	2	189	0
Malav	Harpur	215	983	210	768	5	94	0
Barigaon	Barigaon	438	1592	430	1270	8	158	0
Total		**1629**	**6315**	**1611**	**4974**	**20**	**720**	**1**
Coverage		–	–	**98.9**	**78.8**	–	–	–
Bhathat block								
Jungle Dumri-2	Bangla	293	1044	263	839	30	85	0
Dumri-1	Aurahiya	337	1220	312	1027	25	115	0
Bhathat	Tarkulha	301	951	293	804	8	58	0
Gulhariya	Hafiznagar	315	1013	264	959	51	47	0
Ghoradaur	Pokharbhinda	365	1097	345	939	20	24	0
Chakiya	Chakiya*	226	843	198	680	28	44	0
Total		**1837**	**6168**	**1675**	**5248**	**162**	**373**	**0**
Coverage		–	–	**91.2**	**85.2**	–	–	–

Entomological evaluation: Evaluation of entomological parameters was continued in the intervention {Belghat (IRS) and Campierganj (LLIN)} and the control (Majhgawa) blocks. Fortnightly indoor (human dwellings, cattle sheds and mixed dwellings) and outdoor (peri-domestic) resting collections were carried out in the study villages, Bahadurpur, Bharsi, Barigaon, Harpur and Gayghat in Belghat block, Sarpatha, Machligaon, Ramnagar, Shivpur and Kaharpurwa in Campierganj block and Katrari, Pidara, Lahilparkhas, Babhani and Saraura in Majhgawa block. Unfed female mosquitoes were pooled for determining the minimum infection rate (MIR) and stored in -80°C. Blood meal samples from fully engorged females were taken on filter paper for determining human blood index (BMI). Mosquito samples were transported to VCRC laboratory for processing and analysis.

Vector density (PMD): The resting density (number per man-hour) of the vector species, *Culex tritaeniorhynchus* in the intervention blocks, Campierganj & Belghat and in the comparison block, Majhgawa is given in Figure 1.26. During the period of four years from 2013 to 2016, the vector density in relation to time (seasonality)

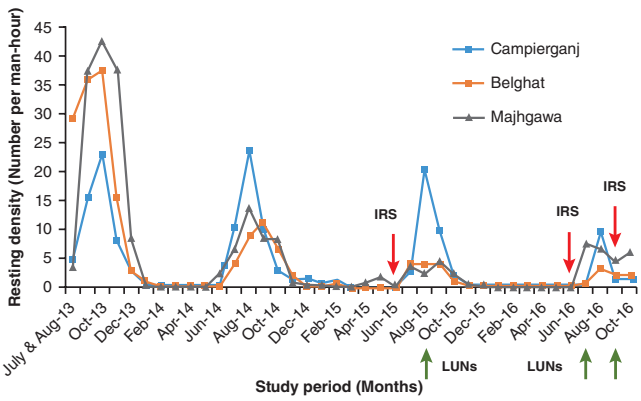


Figure 1.26 **Resting density of Culex tritaeniorhynchus in intervention (Campeirganj- LLIN, Belghat- IRS) and control blocks (Majhgawa)**

exhibited a similar trend in all the three blocks with higher densities during July to October. The density peaked during September-October in 2013, August-September in 2014 and 2015, and July-August in 2016. Resting density of *Cx. tritaeniorhynchus* indoors was relatively higher than outdoors in the intervention and the control blocks. The density of the other two vector species, namely, *Cx. vishnui* and *Cx. pseudovishnui* was very low or almost negligible in the three blocks.

Post-intervention evaluation – 2015: As reported in the Annual Report 2015, after the indoor residual spraying in June 2015, the resting density of *Cx. tritaeniorhynchus* was lower in Belghat Block than the density recorded during the same period in the previous year (Figure 1.26). Although, the density in the comparison block, Majhgawa remained comparable till September 2015, the density recorded in Belghat in October 2015 was lower than that of Majhgawa.

Post-intervention evaluation – 2016: Following the second and third IRS in June 2016 and September 2016, respectively, there was a significant reduction in the density of the vector species in Belghat block from July to October 2016 (the peak season) not only compared to the density recorded during the same period in the previous years, 2015, 2014 and 2013, and also when compared to the density in the control block Majhgawa (Fig. 2). In Campierganj block, where LLINs are distributed, the vector density was lower during July to October 2016 when compared to the density recorded during the same months in the previous years. Also, the vector density recorded in this block during September and October 2016 was markedly lesser than the density in the comparison block, Majhgawa. However, all the Sub-Centres in Campierganj block are not yet covered with distribution of LLINs and distribution is expected to be completed by October/November 2016. Only after this, the impact of this intervention measure could be fully realized. Hence, it is necessary to continue the study for one more year. Further, parallel post-intervention data to assess the effectiveness of the two intervention measures, IRS and LLIN, will be available only if evaluation is continued from November 2016 to October 2017.

Blood Meal Index (BMI): During the post-intervention period (July 2015 to September 2016), a total of 566 blood meal samples of *Cx. tritaeniorhynchus* were collected from the three blocks (two intervention and one control block) for identification of source of feeding and out of this 302 samples were analysed. The BMI for human was 0.016 in Belghat, 0.057 in Campierganj and 0.012 in Majhgawa block, indicating a low preference of the vector species to feed on human.

Minimum Infection Rate (MIR): A total of 1,011 *Cx. tritaeniorhynchus* females (in 171 pools), 212 from Belghat and 399 from Campierganj (intervention blocks) and 400 from Majhgawa (control block) were collected for JE virus detection. RT-PCR assay is being performed on these samples.

Further work to be carried out: In Campierganj block, another 35,000 LLINs are to be distributed and post-intervention evaluation needs to be continued to cover the next transmission season i.e. up to October 2017 to assess the impact of LLIN and parallel evaluation has to be carried out in IRS (Belghat) and comparison block (Majhgawa).

1.4.7. 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

Pradeep Kumar N, Srinivasan R, Natarajan R, Shazia wafai, and Jambulingam P

Objectives:

- To document the mosquito fauna of the Himalayan region 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.

In the earlier mosquito survey in 2015 in the state of J&K, a total of 34 species was recorded of which 7, 5 and 2 species were first time records in Ladakh, Kashmir and Jammu division respectively. *Culex. pipiens*, *Coquillettidia richiardii* and *Ochleoratatus caspius* are the new country record for India. The progress made during the reporting period is given below.

Survey was carried out during the months of July and August 2016 in Jammu & Kashmir and Himachal Pradesh to document mosquito species diversity. A total of 27 collections (adult and immature) were carried out in 97 localities in Jammu, Somba, Kathuva, Udhampur, Kargil and Leh districts of J&K and three districts of Himachal Pradesh namely Kullu, Solan and Shimla (Figure 1.27).

Altitudes and other geo-coordinates are documented for all the collection sites. The altitude in Jammu division ranged from 260m to 656m and in Ladakh division from 2715m to 4795m, while the altitude ranged from 860m to 3930m in Himachal Pradesh.

A total of 2,672 adult mosquito specimens were collected during the survey. Of which 1,588 specimens were pinned and 1,084 were preserved

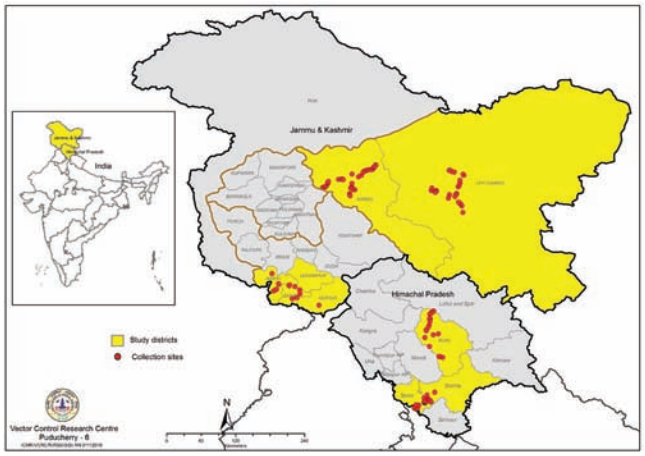


Figure 1.27 Map showing the study districts and collection sites

in stock vials. A total of 515 larvae, larval and pupal skins were mounted on slides. Only 519 specimens from Jammu and Ladakh division were examined, in which 42 species of mosquitoes were identified. Among them 8 species were the time reports in Jammu division and two anopheline species namely, *An. gigas* and *An. barianensis* were reported first time from Ladakh division. Remaining specimens are under examination.

DNA Barcoding: A total of 107 specimens were submitted for molecular confirmation from the earlier collection. Sixty six specimens belongs to 26 species were DNA Barcoded and the remaining are under process. The barcoding of the samples matched with the morphological identification except for two species namely, *Cx. viridiventer* and *Cx. pallidothorax* which were grouped together into a single taxonomic clade (Figure 1.28). Also, *Cx. theileri* was found to be a complex of at least 2 morphologically similar species. These observations require further investigations.

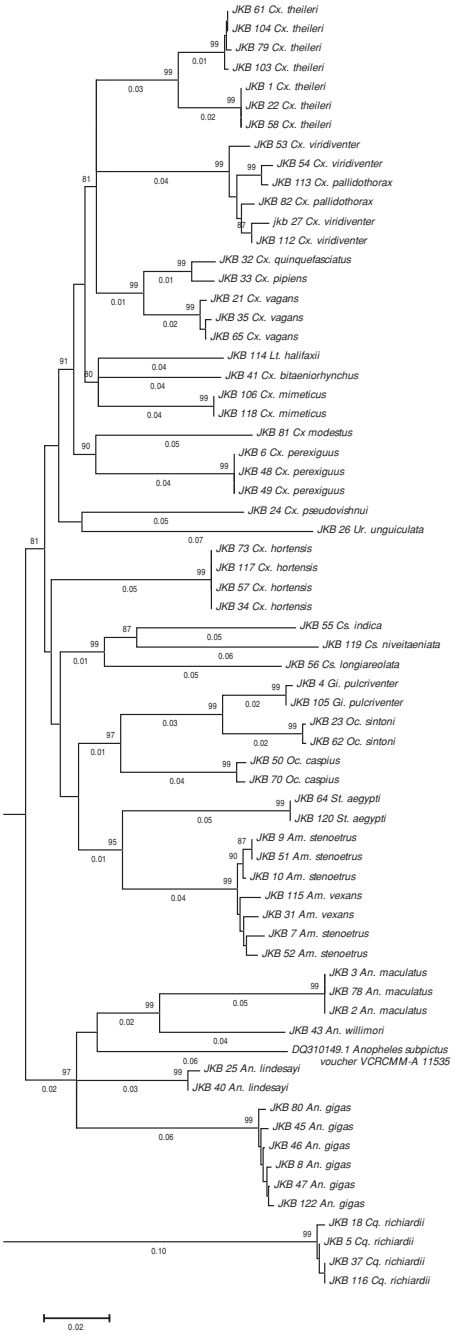


Figure 1.28 DNA Barcode analysis of mosquito specimens collected from Himalayan region

1.4.8. Vector surveillance for ZIKV in selected high risk areas of India

EM 1603: Jul 2016 – Jun 2017

Pradeep Kumar N, MuthuKumaravel and Jambulingam, P
Collaborating Institutions: National Institute of Malaria Research, Centre for Research in Medical Entomology, Madurai and Directorate of Health Services, Govt. of Kerala

Recent outbreak of ZIKV in Brazil and subsequently to about other 66 countries globally affecting about 1.8 million is a major setback in the global health scenario. This comparatively less virulent Flavivirus has evolved to cause major complications as microcephaly in newborns (born to infected mothers during pregnancy) and Galline Barre Syndrome during the present outbreak. Its transmission recorded during the recent outbreak on sexual contact and on contact with body fluids of the patient has caused much concern. World Health Organization declared a global public health emergency calling all the countries to be on high alert of this disease early in February, 2016. The Ministry of Health and Family Welfare, Govt. of India has notified and ICMR has identified VCRC, CRME and NIMR to carry out intensive

vector surveillance for ZIKV, in collaboration with respective state Health Depts.

Objectives:

- To collect mosquito samples in selected high risk areas of Dengue
- To standardize RT-PCR assay for detection of ZIKV in mosquitoes
- To screen the mosquito samples in pools for ZIKV
- To share the results and review the situation with Health authorities

Study Areas:

VCRC – Thiruvananthapuram, Kottayam, Ernakulam and Mallapuram (Kerala state) and in Puducherry
CRME – Chennai, Madurai, Kanyakumari and Tirunelveli Districts of Tamil Nadu
NIMR – Delhi and Bengaluru Corporations

A hands-on training program of entomologists of 4 Districts viz, Thiruvananthapuram, Kottayam, Ernakulam and Mallppuram was held at VCRC field unit Kottayam on SOP of collection process and processing of samples to be send to VCRC for ZIKV virus infection detection during 3th March, 2016. About 24 personnel participated in the training program. As per the protocol, the district Vector Control Units collect samples of *Aedes* mosquitoes from high risk villages in their districts and identify those and store in pools in TRI reagent supplied to them in 2ml Eppendorf tubes. The pools of mosquitoes are brought to VCRC laboratory on Wednesdays every week. The materials required for the same was distributed o the DVC units. In addition to these, 8 villages in Puducherry were selected and the entomology team collected samples and are brought to VCRC for processing.

The methodology adopted for detection of infection is as follows: The specimens brought to VCRC laboratory was processed for extraction of RNA and was followed by multiplex RT-PCR for detection of infection with ZIKV /DENV. Initially, 2 DNA primers (Balm *et al.*, 2002 & Faye *et al*, 2008) were screened for the study. However, as the first set of primers yielded a few non-specific amplifications in some reactions, the more specific second set of primers was selected. This set of primers used amplify 364 bp of the envelope gene of ZIKV (Faye *et al.*, 2008). This was integrated with and the set of DNA primers which amplify 511 bp of CprM gene of DENV (Lanciotti et al., 1992), as the annealing temperatures were found to be same for both. Any mosquito pool yielding PCR amplified fragments for ZIKV was further processed by Real Time PCR using Real star RT-PCR for confirmation. Also the fragment amplified was sequenced for genetic analysis of the strain of the virus involved.

Altogether, in Kerala state, 4040 Aedes mosquitoes (*Ae. aegypti* -1389; *Ae albopictus* – 2176 & *Ae. vittatus* – 475) were processed in the study in 939 pools. These specimens included those were collected and send to VCRC Field unit, Kottayam, by District Vector Control units from Thiruvananthapuram (987), Kottayam (1862), Ernakulam (544) and Mallappuram Districts (470) and those collected by VCRC from areas in Alappuzha (80) and Pathanamthitta (94) Districts from where Dengue cases were reported during the period of study. From Puducherry, 180 specimens of *Ae.aegypti* were collected and processed in 26 pools so far.

None of these mosquito specimens (n=4240) processed were found infected with ZIKV, ruling out active transmission of ZIKV from the high risk villages/urban areas selected in the study. The studies are ongoing.

1.4.9. Preliminary studies on Kyasanur Forest Disease virus in ticks and antibodies in rodents in potential risk areas of adjoining States to Karnataka

IM 1407: Apr 2014 – May 2016

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

Kyasanur forest disease (KFD) is an emerging zoonotic tick-borne viral haemorrhagic fever caused by KFD virus (KFDV), first reported from Kyasanur forest in Shimoga district of Karnataka in 1957 and has been confined to five districts of Karnataka State until 2010. During recent years (2012–2015), incidence of human cases of KFD/monkey deaths has been reported from newer areas of Karnataka and adjoining states such as Chamarajanagar district of Karnataka, Malappuram and Wayanad districts of Kerala, Nilgiri district of Tamil Nadu and Sattari taluk of Goa. Therefore, a survey was carried out to detect the virus in ticks and rodent reservoirs in potential risk areas of Karnataka and adjoining states and to determine the cause of the recent upsurge and emergence of KFD in the newer areas.

Objectives:

- To investigate the presence of KFD virus in ticks and antibodies in rodents from potential risk areas of Karnataka and adjoining States.

- To determine the environmental risk factors that favours the circulation of KFD virus in the above potential risk areas.

Summary of the work done:

Collection of ticks species was carried out in Thirthahalli taluk of Shimoga district and Mulehole forest range of Chamarajnagar district of Karnataka where outbreak of human cases of KFD was reported in 2014, Pulpally and Sulthan Bathery forest ranges of Wayanad district, Kerala where outbreaks of human KFD cases were reported in 2014–2015, Nilambur and Nedumkayam reserve forests (Nagamalai hills) in Malappurram district of Kerala where KFD virus in both humans and monkeys was detected in 2014, four sites at Mudumalai Tiger reserve in Nilgiri district, Tamil Nadu where KFD virus was detected from autopsy of dead monkeys in 2013 and two sites at Sathyamangalam reserve forest in Erode district of Tamil Nadu where evidence of KFD virus circulation was not reported until 2015 (Figure 1.29). Epidemiological investigation was also carried out in Malappuram and Wayanad districts of Kerala. In each of the selected site, ticks from the forest floor were collected using lint clothes (100 x 70 cm) by flagging method.

A total of 6738 tick specimens belonging to eight species of genus *Haemaphysalis*, two species each of genus *Amblyomma, Boophilus,* and *Rhipicephalus* and one species each of genus *Ixodes* and *Hyalomma* was collected (Table 1.15). In all the districts surveyed *Haemaphysalis spinigera,* the primary vector of KFD virus was the predominant tick species collected. The percentage of *H. spinigera* ranged from 39.0% (Malappuram) to 97.5% (Shimoga) in the districts surveyed. *Haemaphysalis turturis,* the other major vector of KFD virus (KFDV) was also recorded in all the districs surveyed which constituted 11.6% of the total tick specimens collected. Other *Haemaphysalis* species collected were *H. bispinosa, H. intermedia, H. cuspidata, H. wellingtoni, H. aculeata* and *H. kinneari*. *Amblyomma* species formed 4.7% of the total ticks collected.

A total of 4418 tick samples (pooled in 213 vials) were screened by RT-PCR at NIV, Pune for evidence of KFD virus infection. Two pools, one from Thirthahalli taluk, Shimoga district and the other from Mudumalai, Nilgiri district was positive for KFD virus by RT-PCR assays.

In addition, collection of rodents was done from Thirthahalli taluk of Shimoga district and Mulehole forest range of Chamarajnagar district of Karnataka using Sherman traps to determine antibodies against KFDV. Thirteen rodents belonging to six species were trapped. From the trapped rodents, blood, liver, lung and kidney tissue samples were collected and screened by RT-PCR and IgG ELISA. None of the samples showed positive for KFDV infection.

Incidence of human cases of KFD/monkey deaths, high abundance of *Haemaphysalis* vectors and KFDV infection in tick vectors indicate that the districts have become vulnerable to KFD outbreaks. Preventive measures (vaccination of high risk groups) coupled with intensive health education should be carried out prior to transmission season. Investigation showed

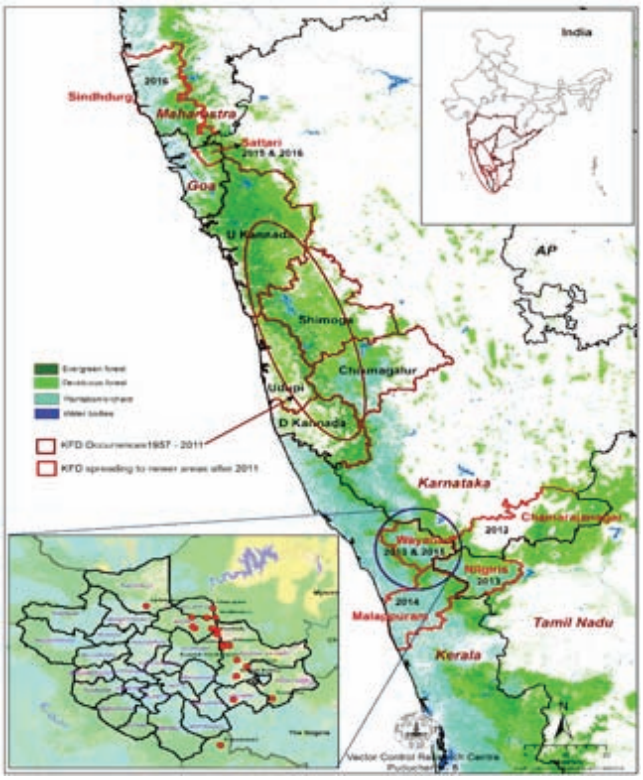


Figure 1.29 Map showing the study districts in Karnataka, Tamil Nadu and Kerala

TABLE 1.15

Species diversity of Ixodid ticks collected from different districts of Karnataka, Kerala and Tamil Nadu

Sl. No.	Species	Districts						
		Shimoga	Chamraj-nagar	Wayanad	Nilgiri	Erode	Malappuram	Total
1	*Ambylomma integrum*	0	0	0	0	0	4	**4**
2	*Boophilus annulatus*	0	0	0	0	0	4	**4**
3	*Boophilus microplus*	0	0	42	0	0	0	**42**
4	*Haemaphysalis spinigera*	231	197	1442	914	519	468	**3771**
5	*Haemaphysalis turturis*	2	12	486	110	81	94	**785**
6	*Haemaphysalis bispinosa*	1	3	429	8	0	198	**639**
7	*Haemaphysalis cuspidate*	1	0	5	0	0	3	**9**
8	*Haemaphysalis intermedia*	2	1	2	23	0	0	**28**
9	*Haemaphysalis aculeate*	0	0	5	0	0	2	**7**
10	*Haemaphysalis papua kinneari*	0	0	0	0	0	1	**1**
11	*Haemaphysalis wellingtoni*	0	0	57	0	0	0	**57**
12	*Rhiphicephalus haemophysaloides*	0	0	1	0	0	0	**1**
13	*Ambylomma* sp.	0	9	104	88	29	85	**315**
14	*Haemaphysalis* sp.	0	0	218	368	135	313	**1034**
15	*Hyalomma* spp.	0	0	1	0	0	0	**1**
16	*Ixodes* sp.	0	0	0	0	0	15	**15**
17	*Rhiphicephalus* sp.	0	0	6	14	0	5	**25**
	Total	**237**	**222**	**2798**	**1525**	**764**	**1192**	**6738**

that a particular population belonging to the Kattunayakan triabl community is at risk because of their vocational movement to the forest.

1.4.10. Prevalence of scrub typhus vectors/ rodent hosts and the pathogen, *Orientia tsutsugamushi*, in areas reporting human cases of AES in Gorakhpur district, Uttar Pradesh

EM 1601: Jun 2016 – May 2017

Sadanandane C, Paily KP, Pradeep Kumar N, Elango A, Athisaya Mary K, and Jambulingam P

Outbreaks of acute encephalitis syndrome (AES) with unknown etiology are reported every year in Uttar Pradesh, particularly in the Gorakhpur district. Though, initially, Japanese encephalitis (JE) was considered as the major cause of the problem in the region, analysis of AES surveillance data indicated that only 8% of the cases were due to JE. Recent reports, based on serological and molecular biological results, indicate that about 60% of the AES cases are positive for scrub typhus infection. In view of this, ICMR has initiated a comprehensive multi-disciplinary investigation to generate epidemiological, entomological and socio-behavioural evidence on the endemicity of scrub typhus infection in Gorakhpur district. Vector Control Research Centre, Puducherry is entrusted with the study of entomological and zoonotic aspects of scrub typhus transmission in this endemic area.

Objectives:

- To study the prevalence of scrub typhus vector mites and rodent hosts in areas reported with human cases of AES in Gorakhpur, UP.
- To determine *Orientia tsutsugamushi* infection in the vector mites/rodent hosts prevalent in the areas reported with human cases of scrub typhus.

A longitudinal survey of rodent hosts and trombiculid mites, the vectors of scrub typhus, is carried out in eight villages of Gorakhpur district (from six blocks viz., Bhathat, Pipraich, Jungle Kouria, Chargawan, Bramhpur and Khorabar), where cases of AES were reported recently (Figure 1.30). Trombiculid mites are collected directly from rodents/shrews trapped by using Sherman live traps (7.6 x 8.9 x 22.9 cm). The traps are set in peri-domestic areas one hour prior to sunset and retrieved the next day morning. The trapped rodents are anaesthetized and identified after recording their morphological characteristics.

The ecto-parasitic mites are collected by combing the rodents against the fur of the rodents over a white enamel tray and the mites were preserved in 75% ethanol until mounted on slides. After mounting on microslides the mites are identified up to species level following standard taxonomical keys. Samples of preserved mites are subjected to Nested PCR for detection of the scrub typhus pathogen (*O. tsutsugamushi*) specific gene encoding 56 kDa protein. Blood samples of the trapped rodents/shrews are collected after euthanasia for detection of scrub typhus pathogen through PCR assay and antibodies through Weil-Felix test. In the antibody detection test using sera samples, antibody titres against the OxK antigen, which is specific to *O. tsutsugamushi*, are determined. DNA extracted from Blood samples are subjected to PCR for detection of the presence of *O. tsutsugamushi* specific gene encoding 56 kDa protein as well as 60 kDa protein.

A total of 553 rodents/shrews were trapped using 2788 Sherman traps, set in the selected study villages. The overall trap rate recorded was 19.1%. Of the total 533 rodents trapped, 126 were trapped dead and the remaining 281 were screened for trombiculid mites. About 61% rodents/shrews were infested with trombiculid mites. *Suncus murinus*, the Index animal of scrub typhus was the predominant species (75.1%) followed by *Mus booduga* (17.7%), *Rattus sp.* (6.7%) and *Mus musculus* (0.6%).

A total of 3674 mites belonging to 14 species of trombiculids were recovered from the trapped rodents/shrews (Table 1.16). *Leptotrombidium (L) deliense*, the established vector of scrub typhus in India was the predominant species (76.1%). Followed by this were *Schoengastiella ligula* (12.0%) and *Walchia gujaratensis* (5.1%). Overall, the number of trombiculid mites infested

***Leptotrombidium deliense*, the vector of Scrub typhus pathogen**

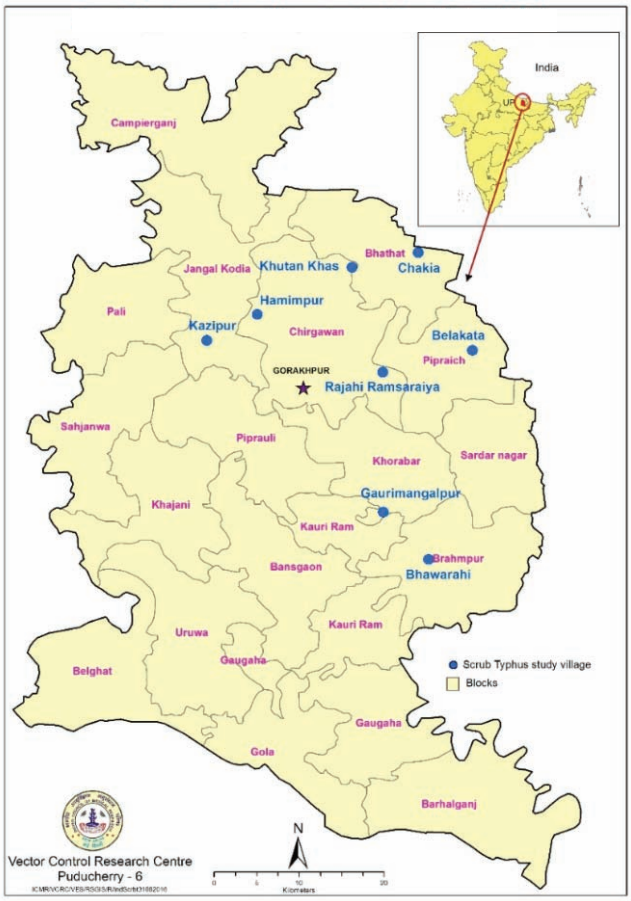


Figure 1.30 Scrub Typhus study villages in Goralkpur Distruct (UP)

TABLE 1.16

Species diversity of trombiculid mites collected in Gorakhpur district, UP

Sl. No.	Mite species	Number collected	Percentage
1	*Leptotrombidium deliense*	2794	76.1
2	*Schoengastiella ligula*	440	12.0
3	*Walchia gujaratensis*	187	5.1
4	*Schoutedenichia nagpurensis*	92	2.5
5	*Schoengastilla ceylonica*	69	1.9
6	*Schoengastilla punctata*	16	0.4
7	*Ascoschoengastia Indica*	14	0.4
8	*Trombicula hypodermata*	14	0.4
9	*Leptotrombidium insigne*	9	0.3
10	*Schoengastiella argalea*	7	0.2
11	*Herpetacarus schlugeri*	4	0.1
12	*Walchia ewingi*	4	0.1
13	*Schotendenichia capillata*	2	0.1
14	*Schoengastiella* sp.	13	0.4
15	*Schoengastia* sp.	4	0.1
16	*Leptotrombidium* sp.	3	0.1
17	*Walchia* sp.	2	0.1
	Total	**3674**	

per rodent/shrew was 10.7. The number infested per rodent/shrew was higher with *Rattus* sp. (16.0) followed by the shrew mouse, *Suncus murinus* (12.3) (Table 1.17). The Chigger *(L. deliense)* index ranged from 0.60 to 79.3 in different villages surveyed. Except in one village, the Chigger *(L. deliense)* index was well above the critical level of 0.69 per rodent/shrew in the surveyed villages.

Detection of Orientia tsutsugamushi DNA in chigger mites: A total of 1421 mite samples pooled in 168 vials were screened for detection of *O. tsutsugamushi* DNA through nested PCR. Of 168 pools, seven pools were positive for the gene encoding 56 kDa protein and its identity was confirmed as that of *O. tsutsugamushi* by DNA sequencing. Presence of *O. tsutsugamushi* was detected through PCR in mite samples belonging to *L. deliense*. All the infected *L. deliense* specimens were collected from shrew mouse, *Suncus murinus*.

Screening of rodent/shrew blood samples for Orientia tsutsugamushi: A total of 114 sera samples collected from rodents/shrews were tested for the presence of antibodies against *O. tsutsugamushi* using OxK antigen through Weil-Felix test and 57.0% of them were positive with an antibody titre of 1:160 (Table 1.18). Among the samples tested, 8.0% showed positive reaction to antibodies against Ox-19 (*Rickettsia typhi*) and 28.0% against Ox-2 (*R. conorii*).

Blood samples collected from 142 rodents/shrews were analyzed through PCR for detection of scrub typhus specific genes encoding 56 kDa and 60 kDa proteins. One rodent sample was positive for the gene (483 bp) encoding 56 kDa protein (Figure 1.31) and 25 were positive for gene (300 bp) encoding 60 kDa heat shock protein (Figure 1.32). Identities of these genes were confirmed as that of *O. tsutsugamushi* through DNA sequencing. DNA sequences obtained were submitted to Gene Bank and accession numbers obtained. Both antibody positive reaction as well as PCR positivity was highest among the known index animal of scrub typhus, *S. murinus*.

These findings along with the observation of predominance of *Suncus murinus*, compared to other species of rodents, indicate the possible

TABLE 1.17

Infestation of mites per rodent/shrew (chigger index)

Rodent/shrew species	Number of rodents/ shrews collected (%)	No. of mites recovered	No. of mites/ Rodent	No. of *L. deliense* collected	No. of *L. deliense*/rodent
Suncus murinus	259 (75.1)	3192	12.3	2540	9.8
Mus booduga	61 (17.7)	112	1.9	29	0.5
***Rattus* sp.**	23 (6.7)	369	16.0	225	9.8
Mus musculus	2 (0.6)	0	0.0	0	0.0
Total	**345**	**3674**	**10.7**	**2794**	**8.1**

TABLE 1.18

Prevalence of rickettsial pathogens in different species of rodents/shrews collected from AES reported villages of Gorakhpur district, UP, as diagnosed through Weil-Felix test and PCR

Rodent species tested Weil-Felix test	% +ve for *O. tsutsugamushi* (Ox-K)	% +ve for *R. typhi* (Ox-19)	% +ve for *R. conorii* (Ox-2)	% +ve for antibody (against Ox-K, Ox-19 & Ox-2)	Rodent species tested through PCR	% +ve for *O. tsutsugamushi* 60kDa protein	% +ve for *O. tsutsugamushi* 56kDa protein
Suncus murinus (n = 110)	57.3	7.3	28.2	1.8	***Suncus murinus*** (n = 133)	18.0	0.8
***Rattus* sp.** (n = 3)	66.7	33.3	33.3	33.3	***Rattus* sp.** (n = 3)	0.0	0.0
Mus booduga (n = 1)	0.0	0.0	0.0	0.0	***Mus booduga*** (n = 6)	16.7	0.0
Total (n = 114)	**57.0**	**7.9**	**28.1**	**2.6**	**Total** (n = 142)	**17.6**	**0.7**

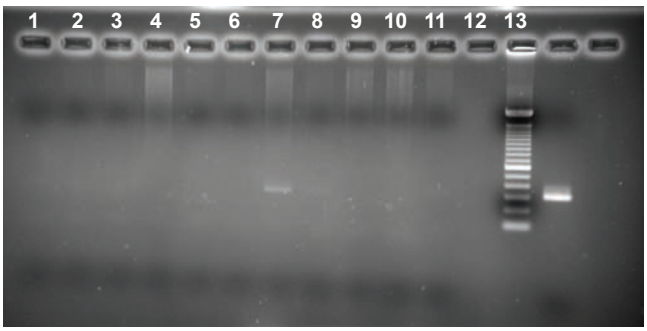


Figure 1.31 Nested PCR amplication of 483 bp of *O. tsutsugamushi* in rodent blood samples collected from Gorakhpur, UP (Lane 1-4 & 6-10 negative samples, Lane 5: positive sample, Lane 11: negative control, Lane 12: positive control, Lane 13: 100 bp ladder)

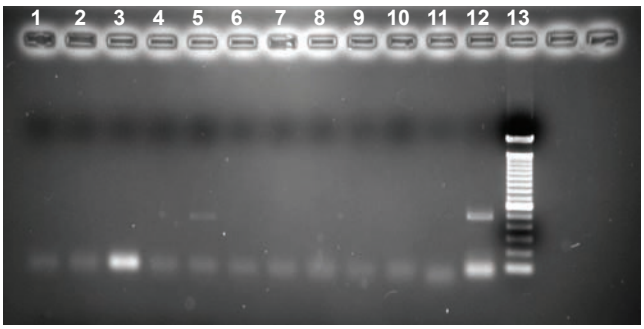


Figure 1.32 PCR amplification of 300 bp of *O. tsutsugamushi* in rodent blood samples collected from Gorakhpur, UP (Lane 1-6 & 8-11 negative samples, Lane: 7 positive samples, Lane 12: negative control, Lane 13: 100 bp ladder, Lane 14: positive control)

role of this animal as the maintenance/reservoir host of the pathogen in the study villages selected at Gorakhpur, where human cases of scrub typhus are reported. The study is being continued in all the eight study villages selected for collection and analysis of more number of both rodent and mite samples for further confirmation of the results and to determine the seasonality of host/vector prevalence and parasite infection.

1.5 MICROBIAL / CHEMICAL AGENTS FOR VECTOR / PARASITE CONTROL

1.5.1. Optimization of upstream and downstream process for the production of mosquitocidal metabolite(s) by an indigenous bacterium *Bacillus amyloliquefaciens* (VCRC B483) and assessment of its anti-microbial activity

IM 1302: Apr 2013 – Mar 2016

Manonmani AM, Geetha I, Mathivanan A, Parija SC, (JIPMER)
Collaborating Institute: Department of Microbiology, JIPMER, Puducherry

Bacillus amyloliquefaciens (VCRC B483), an indigenous bacterium isolated from mangrove forests of Andaman & Nicobar Islands was found to produce secondary metabolite(s) exhibiting mosquitocidal and antibacterial activity. The present project was taken up with the following objectives.

Objectives:

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

Production of the metabolite was standardized by varying the "C" and "N" source and a new medium was designed. Downstream process was optimized for maximum recovery of the metabolite from the culture supernatant. Mosquito larvicidal and pupicidal activity of the crude metabolite was determined using immature stages of *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. The genes responsible for the antimicrobial activity of the metabolites of B483 viz., Bacillomycin, Iturin, Bacilysin, Macrolactin, Bacillaene and Difficidin were amplified (Annual Report 2014 & 2015).

During the reporting year, the partially purified metabolite(s) was tested against clinical isolates (n=30) of Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant *Enterococcus* (VRE) and Resistant *Streptococcus pneumoniae* (RSP) in quadruplicate and MIC was determined for the same (Figure 1.33). Antibiotic susceptibility pattern of the clinical isolates to metabolites of VCRC B483 was found to be RSP < VRE < MRSA and MIC of the metabolite follows the pattern of MRSA < VRE < RSP.

Methanolic extract of metabolites of VCRC B483 was analysed by MALDI-TOF (Figure 1.34). The main peaks obtained through MALDI-TOF analysis were indicative of isoforms of *Bacillomycin* D (1031.06, 1067.65, and 1081.77), Surfactin (1044.78,

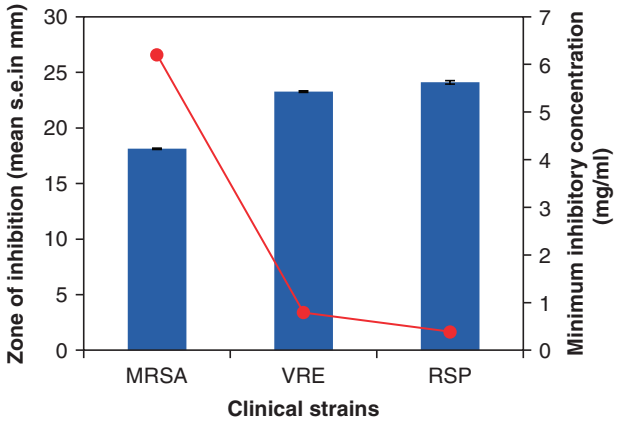


Figure 1.33 Antibiotic susceptibility & MIC of multidrug resistant clinical isolates of MRSA, VRE and RSP to metabolites of *B. amyloliquefaciens* (VCRC B483)

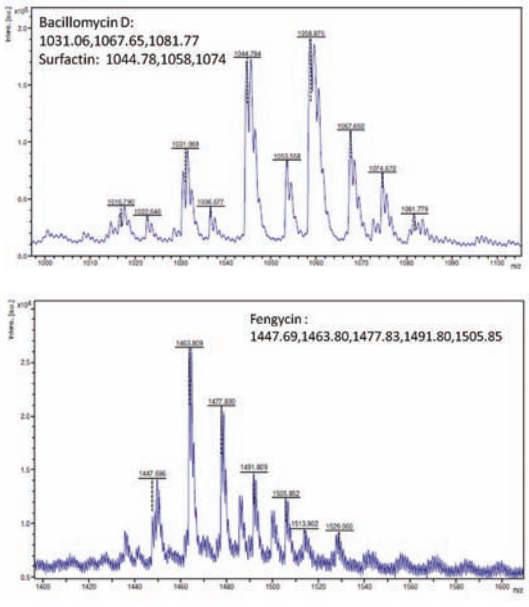


Figure 1.34 MALDI-TOF mass spectrum of the partially purified metabolite(s) produced by *B. amyloliquefaciens* showing isoforms of *Bacillomycin* D, Surfactin and Fengycin

1058.87, and 1074.67) and Fengycin (1447.69, 1463.80, 1477.83, 1491.80, and 1505.85).

Partially purified metabolites were tested against the pupal stages of *An. stephensi* (Table 1.19). The activity of the partially purified metabolite was 6.28 times higher than that of acid precipitate and 1.95 times higher than the methanol residue.

Partially purified metabolites (3 mg) was tested for antifungal activity against phyto pathogens, *Fusarium sp.* (F25) and *Curvularia* sp. (F26) (Plate 1). Zone of inhibition observed for *Fusarium* sp and *Curvularia* sp was found to range between 2 - 7.5 mm and 4 - 9.5 mm respectively. The antimicrobial peptide genes viz., Iturin, Bacilysin, Macrolactin, Bacillaene and Difficidin have been sequenced.

Hence, the metabolites produced by *B. amyloliquefaciens* are versatile in their biological activity as they were found to be effective against mosquitoes vectoring diseases, multidrug resistant clinical bacterial strains and phyto pathogenic fungi.

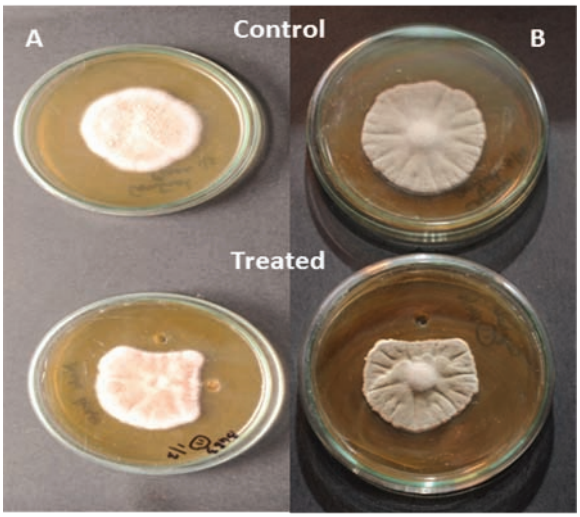


Plate 1 Antifungal activity of *B. amyloliquefaciens* (VCRC B483) against fungal strains *Fusarium* sp. (F25) (A) and *Curvularia* sp. (F26) (B)

1.5.2. 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

The technology related to the Aqueous Suspension (AS) formulation prepared using the *Bacillus thuringiensis* subsp. *israelensis* (VCRC-B17) biomass produced using soya based medium has been patented. In this project, we propose to screen cheaper raw materials for production and design formulations with improved residual activity. As the AS formulation is expected to be used in mosquito control programmes shortly, it is essential to have a DNA fingerprint of this indigenous bacterium.

Objectives:

- To identify alternative, cost effective raw materials for production
- To develop formulations with longer residual activity
- To develop DNA fingerprints 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

Media optimization studies carried out on designing new production media for the mosquitocidal toxins of *Bti* had resulted in 6 media combinations (AR 2015). During the current year, further studies were carried out with the above 6 indigenously designed media and KL9 medium based on horsegram (*Macrotyloma uniflorum*) and KF10 medium based on broken Bengal gram (*Cicer arietinum*) were found to be the best. Testing of the lyophilized cells obtained from KL9

TABLE 1.19

Effect of the metabolite(s) of *B. amyloliquefaciens* in terms of LC_{50} and LC_{90} (µg/ml) on the pupal stage of *A. stephensi*

Test material	LC_{50} (µg/ml)	95% FL	LC_{50} (µg/ml)	95% FL
Acid precipitate (AP)	4.40	(3.94–4.89)	12.90	(10.72–15.65)
Methanol residue of AP	1.37	(1.18–1.52)	2.76	(2.55–3.05)
Partially purified	0.70	(0.67–0.85)	1.70	(1.6–1.9)

and KF10, showed that both the media proved to be almost similar in mosquitocidal toxin production and cost (Table 1.20).

The Bti (VCRC B17) was mass produced in KF10 and biomass obtained was used in the preparation of tablet formulation. This formulation was prepared by mixing the dry lyophilized cell mass of *Bacillus thuringiensis* subsp *israelensis* with ingredients like disintegrant, filler, glidant and lubricant (Plate 2). The formulation was air dried at room temperature and is yet to be tested in the laboratory and simulated field conditions.

Taxonomic markers genes, 16s rRNA, 23s RNA, *gyrA* and *rpoB* respectively and endo-toxin genes, cry4Aa and cry4Ba were amplified from genomic and plasmid DNA using specific primers (AR 2015). Complete nucleotide sequence of 16s rRNA (1556 bp) and *gyrA* (1923 bp) of VCRC B17 strain have been generated. The complete nucleotide sequence of the taxonomic marker genes, 23s rRNA (2925 bp) and rpoB (3534 bp) and endo-toxin genes, cry4Aa (3543 bp) and cry4Ba (3411 bp) and were generated using sequencing primers and the contigs were assembled to obtain complete gene sequence. The sequences were blast searched against the GENBANK nucleotide databases with a sequence coverage of 100% and an e-value of 0. Blast search of *rpoB* sequence of VCRC B-17 against GENBANK nucleotide database, resulted in sequences that are 100% identical with other Bti sequences and different from that of other *Bt* serotypes. Therefore, multiple sequence alignment of Bti VCRC B-17 with blast match sequences with a sequence coverage of 100%, an e-vlaue of 0 and sequence identity of 98% (cut off circumscribed for taxonomic species rank using rpoB gene) was performed using Muscle and Bti sequences were found to vary from Bt sequences at 1339th position with a change in nucleotide of T to C (Figure 1.35). This difference in the nucleotide was used for construction of a phylogenetic tree using Neighbour joining method. The distances were computed using Kimura 2-parameter in Mega V.7.0 and the bootstrap consensus tree was inferred from 500 replicates. Phylogenetic tree deduced showed that all *Bti* strains along with VCRC B-17 formed a distinct cluster from that of other *B. thuringiensis* sequences (Figure 1.36). This indicated that *rpoB* gene can be used as molecular/ phylogenetic marker for the identification of *B. thuringiensis*.

TABLE 1.20

Mosquitocidal toxin production and cost comparison in/of the production media KL9 & KF10

Production media	LC_{50} value (in µg/100ml)	LC_{90} value (in µg/100ml)	Cost (Rs/litre)
KL9 (Horse gram)	2.8	5.2	1.40
KF10 (Bengal gram broken)	2.4	4.6	1.48

Plate 2 Tablet formulation of *Bacillus thuringiensis* subsp. *israelensis*

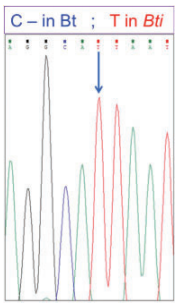


Figure 1.35 Variation in *B. thuringiensis* subsp. *israelensis* at position 1339 Bti-T, Bt-C

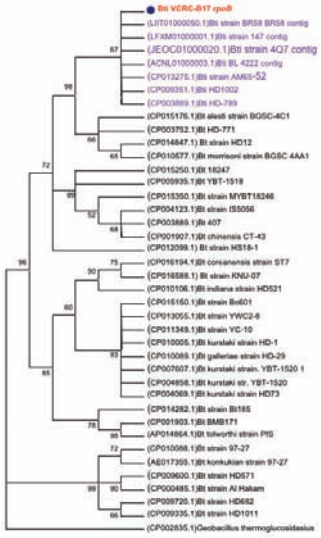


Figure 1.36 Phylogenetic tree constructed with rpoB sequences of Bti VCRC B-17, other Bti and Bt strains show a district cluster with Bti sequences alone

Work to be done:

Using the sequences generated for the endo-toxin genes of VCRC B-17, Bti specific real time PCR will be developed to monitor the persistence of Bti VCRC B-17 in the environment.

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

IM 1404: Apr 2014 – Mar 2017

Nisha Mathew & Jambulingam

Resistance has been reported to each of the major insecticide classes used in the past and present for vector control, including organochlorines, organophosphates and carbamates, and pyrethroids which target sodium channels, acetylcholinesterase, and GABA receptors, respectively, in the insect nervous system. New insecticides with unique modes of action are urgently sought for the management of arthropods that transmit disease-causing agents impacting human health. A new class of insecticides has been reported to be effective against the agricultural pests, the anthranilic diamides, that provides exceptional control through action on a novel target, the ryanodine receptor. The diamides are the most recent addition to the limited number of insecticide classes with specific target site activity that are highly efficacious, control a wide pest spectrum, and have a favorable toxicological profile. Hence, the synthesis of substituted anthranilic diamides for the development of mosquitocidal agents have been taken up with following objectives:-

Objectives:

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

Synthesis was carried out by following standard methods for making 11 anthranilic diamides. Preliminary screening was carried out for all the 11 molecules to assess the mosquito larvicidal activity. From the results DA-9 was found to be effective in killing the 3rd instar larvae of *Culex quinquefasciatus* with moderate effects on *An. stephensi* and *Ae. aegypti* larvae in the preliminary screening (Annual report 2014). Since the yield was found to be low by this method it was slightly modified to improve the yield. This compound was screened at lower concentrations to get the LC_{50} value as 50.67ppm (LCL-UCL 47.58–53.38 and LC_{90} value 87.53 ppm (LCL-UCL 80.04–99.79. From 11 substituted anthranilic diamides we could get a lead molecule (DA-9) with LC_{50} value of 50.67 ppm.

By introducing a link group in the lead molecule the activity was enhanced in such a way that the compound showed larvicidal activity against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* larvae with LC_{50} values of 4.20ppm(LCL-UCL 3.39–5.08), 1.295ppm (LCL-UCL 0.86–1.72) and 19.95ppm (LCL-UCL 16.72–23.12) respectively.

The results shows that this new series of compounds with new mode of action can be explored further by linking with more active moieties to arrive at a promising mosquito control agent as there is widespread development of resistance in mosquitoes to the most commonly used insecticides and not much work is going on in developing public health insecticides.

Outcome: Identified mosquito larvicidal potential of anthranilic diamides with thioamido linkage.

1.5.4. Field Evaluation of pupicidal metabolites of *B. subtilis* subsp. *subtilis* (VCRC B471) and *P. fluorescens* (VCRC B426) against malaria vectors

IM 1608: Apr 2016 – Mar 2018

Manonmani AM, Prabakaran G, Geetha I, Mathivanan A, (Consultant: Balaraman K)

Secondary metabolites of two bacteria viz., *Pseudomonas fluorescens* (B426) and *Bacillus subtilis* subsp. *subtilis* (VCRC B471), were found to be effective on the immature stages of mosquitoes and more importantly, the pupal stages. Pilot scale production technology have been developed and product patents have been granted. In order to translate the technology from laboratory to operational use, it is necessary to evaluate the efficacy of the microbial candidates under field condition, as there are many factors ranging from mosquito strain to breeding habitat characters, which might influence the activity.

Objectives:

- Large scale production of the metabolites of *Bacillus subtilis* subsp. *subtilis* (VCRC B471) and *Pseudomonas fluorescens* (VCRC B426).

- To formulate the metabolites and study their efficacy against pupal stages of *Anophelines* under natural conditions
- To study the stability of the formulations on long term storage

B. subtilis ssp. *subtilis* (VCRC B471) and *P. fluorescens* (VCRC B426) were obtained from the culture collection of Vector Control Research Centre and used in the study. The mosquitocidal metabolites from both these bacterial strains were produced on a large scale and formulated into Aqueous Suspension formulations. The formulations were tested against the vector of malaria, *An. stephensi* in the laboratory as per standard procedures. Yield of the mosquitocidal metabolite produced by VCRC B471 was 7 ± 0.3 g/lit and about 600g was produced during the reporting year. LC_{50} dosage determined for VCRC B471 was found to be 2.1 µl/100ml. Yield of the mosquitocidal metabolite produced by VCRC B426 was 10.4 g/lit and about 1000g was produced during the reporting year. LC_{50} dosage determined for VCRC B426 was found to be 1.4 µl/100ml. The formulations will be shortly taken up for Phase II trials against immature stages of Anophelines breeding in construction sites of Goa. This part of the study is proposed to be carried out with the assistance of the staff of the NIMR field unit at Goa.

1.6 NEW VECTOR CONTROL TOOLS

1.6.1. 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, Baig MM, Subramanian S

MAGNet, a long lasting insecticidal net (LN), complied with the WHO interim specifications (454/LN/2; October 2009) with reference to total content of alpha-cypermethrin, the synthetic pyrethroid used for the treatment, and retention index. MAGNet caused a mosquito knock down (KD) above 95% and/or mortality above 80% for 20 washes; thereby meeting the WHO criteria for the Phase I study (WHO/HTM/NTD/WHOPES/2011.7). MAGNet uses 150 denier high density polyethylene (HDPE) monofilaments, much stronger than 100 denier polyester yarn. In this LN, the insecticide, alpha-cypermethrin, is incorporated within the HDPE filaments and diffuses to the surface slowly (controlled release of insecticide) and this makes a very small percentage of the insecticide sufficient enough to kill mosquitoes available on the surface.

Washing the net removes the insecticide on the surface, but this is replenished by the insecticide within the filaments. The formulation used in MAGNet restores the bio-efficacy within 24 hours and heating is not required to accelerate restoration of bio-efficacy after washing MAGNet; bio-efficacy was reported to be high even after 25 washes. The efficacy of MAGNet was evaluated in experimental huts (Phase II evaluation) against a wild, free flying susceptible population of *Anopheles fluviatilis* sensu lato in terms of mortality, deterrence, blood-feeding inhibition and induced exophily in Odisha State, India following the WHO guidelines/ ICMR Common Protocol.

Objectives:

- The overall objective was to determine the efficacy of MAGNet washed 20 or 25 times relative to the reference LN, Duranet, washed 20 times against susceptible *An. fluviatilis* mosquitoes in experimental huts that simulate local domestic habitations.

Specific objectives:

- To determine the efficacy of unwashed and washed (20 or 25 times) MAGNet in terms of deterrence, induced exophily, blood feeding inhibition and mortality of *An. fluviatilis*
- To record the perceived side-effects of the MAGNet among the volunteers sleeping under the LNs in the experimental huts.

The six identical experimental huts constructed in Kandhaguda village were used for the trial. The huts are specially designed for recording the entering and exiting behavior of mosquitoes and for measuring response to insecticides/ treated nets including mortality.

The trial had six comparison arms: 1) Unwashed MAGNet, 2) MAGNet washed 20 times, 3) Unwashed Duranet, 4) Duranet washed 20 times, 5) Untreated net and MAGNet washed 25 times.

Among the six arms, 7 nets (6 replicate nets for hut evaluation and two additional nets for chemical analysis and cone-bioassay) each of Arm 2 and Arm 4 were washed 20 times and 7 nets of Arm 6 were washed 25 times, taking a regeneration of one day, following the WHOPES washing protocol. The nets were coded to indicate the six Arms (Code X1–X6) and six replicate nets of each Arm (X1A–X1F). Two additional nets in each arm were used for cone bioassays and chemical analysis {Y (before any wash) and Z (after washing cycles) i.e. X1Y, X1Z}. The codes were not communicated to the field staff involved in the hut evaluation. After washing, the nets were shifted to the field site at Malkangiri for experimental hut evaluation.

For hut acclimatization, one adult volunteer slept overnight inside the experimental hut under an ordinary mosquito net for one month. Suitability of the huts in terms of entry of vector mosquitoes comparable to village (tribal) huts, tightness ensuring 80% recovery of released mosquitoes and

absence of scavengers. Clearance from the VCRC ethical committee was obtained to involve human volunteers in the study. A contingency insurance policy was taken for the volunteers.

Species composition: A total of 108 collections, completing three rotations (six weeks to complete one rotation) across all treatments, were done in each of the six experimental huts. Of the total mosquitoes collected (n=922); *An. fluviatilis* formed 16.3%. *An. culicifacies* 16.4%, other anophelines 19.1% and 48.3% was culicines. Since, *An. culicifacies* was resistant to alpha-cypermethrin, further analysis was restricted to *An. fluviatilis,* the major malaria vector in the study area and susceptible to alpha-cypermethrin.

Entry of the vector species in to the huts with treated nets of the five arms was significantly reduced compared to the hut with untreated net ($P<0.05$). Between the five treatment arms there was no significant difference ($P>0.05$). Further, MAGNet washed 25 times did not differ significantly from unwashed MAGNet in terms of deterring hut entry indicating persistent insecticide effect of MAGNet even after 25 washes. The exit rate of the vector species varied from 80.0 to 100.0% in the huts with treated nets against 59.2% in the hut with untreated net, indicating some degree of excito-repellent effect of the insecticide treatment of the nets. Among the treated arms, unwashed MAGNet induced significantly higher exophily than the untreated arm (Table 1.21).

There was 100% blood feeding inhibition with unwashed MAGNet. Further, comparison revealed that the feeding rate was significantly lower with MAGNet washed 20 times and MAGNet washed 25 times ($p<0.05$) compared to the untreated arm. The total mortality (Table 1.21) of the vector species did not differ significantly between the five treated arms including MAGNet washed 25 times indicating wash resistance of MAGNet for longer period without losing insecticidal effect against the vector mosquito.

Cone-bioassay results: Prior to any wash and after 20/ 25 washes all the five treated arms produced 100% mortality, while in the untreated arm the mortality was zero. Similarly, prior to (*An. fluviatilis* was used in cone-bioassays) and at the end of the experimental hut evaluation (*An. jeyporiensis*) all the five treated arms showed 100% mortality of and on the untreated net the mortality was zero.

Perceived side effects: Among the 12 volunteers interviewed, no one reported any perceived side effect including nose irritation, itching of body parts like face and hands etc. The volunteers also stated that while sleeping they did not smell any odour from the nets. They were reportedly benefitted from reduced mosquito bites in the huts and an undisturbed night-sleep throughout the study period.

The performance of MAGNet after 20/25 washes was comparable to unwashed MAGNet as well as to the reference net, DuraNet. Compared to the untreated net, washed MAGNets showed higher efficacy in terms of deterrence and blood feeding inhibition. However, the number of *An. fluviatilis* collected in the control arm was not adequate and therefore the trial is proposed to be repeated completing one or more rotations in the coming mosquito season in order to increase the sample size and make the comparison more valid. Approval of SAC is requested for four months no cost extension of this study from November 2016 to February 2017.

TABLE 1.21

Entry, exit, feeding and mortality of *An. fluviatilis* in treated and untreated arms

Arms	Number of collections	Number entered (entry)	Exit rate (%) (induced exophily)	Feeding rate (%) (feeding success)	Total Mortality (%) (killing)
Unwashed MAGNet	108	15	93.3	0.0	80.0
MAGNet washed 20 times	108	11	90.9	27.3	81.8
Unwashed Duranet	108	6	83.3	50.0	83.3
Duranet washed 20 times	108	5	100.0	20.0	80.0
Untreated net	108	103	59.2	68.0	0.0
MAGNet washed 25 times	108	10	80.0	20.0	90.0

1.7 BIOMEDICAL INFORMATICS

1.7.1. Biomedical Informatics Centre of ICMR (Phase-II)

EM: Jan 2014 – Dec 2019

Pradeep Kumar N, Gunasekaran K, Subramanian S, Nanda Kumar Y & Jeyakodi G

Objectives:

- Development of Vector Informatics Database (VectorInfo: Repository of medically Important Indian Arthropods)
- Lymphatic Filariasis Clinical Data Management System (LFCDMS)
- To study prevalence of arbo-viruses in India with reference to their genomics, meta-genomics and molecular adaptation to vectors.
- Prediction and evaluation of antigenic determinants on proteins of *Wuchereria bancrofti*.

Progress:

Development of Vector Informatics Database (VectorInfo: Repository of medically Important Indian Arthropods) 'VectorInfo' is a digital assembling of medically important Indian arthropod vectors organised from various research articles and available resources. Vector-borne infectious diseases cause a significant fraction of the global infectious disease burden. The aim of this database is to facilitate the vector research community by providing the entire vector scenario in a single window. The database covers all the essential information needed for vectors and vector-borne diseases eradication. Figure 1.37 shows the various information ranging from basic biology to omics data, organised in the database.

The VectorInfo database (Repository of medically important Indian arthropods) design has been organised as biological, omics and insecticides data. Data collection and web design of the biological aspect of each vector species starts from morphology to control mechanism has been completed with the reference. Each species photographs has to be captured. Genomics data (gene name and gene derived molecular information), transcriptomics data (promoter region and promoter) and proteomics data (protein name, 3D structure, physiochemical properties, motifs, allergenic property, etc.,) has been collected from the available databank and the design has been completed. Protein structure has been constructed using I-Tasser server. Collection of enzyme name, function, reaction, and reaction type and pathway information for each vector species is continued. Immunobiological data and insecticides will also be collected and incorporated in the database.

Lymphatic Filariasis Clinical Data Management System (LFCDMS): Lymphatic Filariasis Clinical Data Management System (LFCDMS) is an integrated clinical trial information system for Lymphatic Filariasis (LF) Clinic in Vector Control Research Centre (VCRC). VCRC filarial clinic provides diagnostic, therapeutic and morbidity management services to LF patients reporting from and around Puducherry and field practice areas of Tamil Nadu. LFCDM is designed by PHP, a server-side scripting language with SQL backend database and XAMPP server (Figure 1.38). The system allows for Remote Data Capture (RDC) so that local and remote personnel enter and manage clinical data over a LAN, intranet or the internet with password protection.

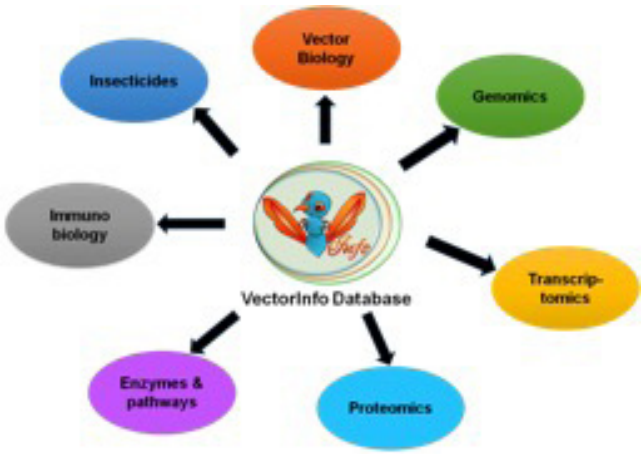


Figure 1.37 'VectorInfo' database data organization

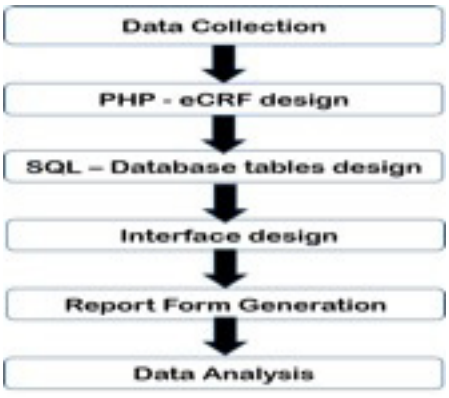


Figure 1.38 The Filarial data management and analysis

The data required for creating Case Report Form (CRF) is collected from the printed form of patient case record available from the VCRC LF Clinic. PHP, a server-side scripting language designed primarily for web development is used to convert the printed CRFs (electronic CRF) into user friendly eCRFs (Figure 1.39). The eCRF provides all the necessary comments and selective options for the user to enter correct data. Data validities are also defined. In LFCDMS, patient ID is very important and it must be unique which is used to locate patient's personal, filariasis history and follow-up visit details. So the patient ID value is coded to generate automatically to avoid the wrong entry. Structured Query Language (SQL), designed for the retrieval and management of data is used for creating LFC database tables. All the necessary constraints are defined for the data fields. Adobe dreamweaver, a tool for creating website is used for creating a user friendly website for LFCDMS. Menu based selection is given for easy browsing. All the required data are retrieved from the tables and organized as a various report forms. Non-filarial and filarial status wise patient's personal details and history details are provided, and the necessary filter options are specified. The follow-up-visit reports, offers the number of visits of individual patient and his/her health status. The follow-up-visits can be retrieved by the various filters patient ID, name and date of visit. The data analysis pie chart for viewing the year wise status of non-filarial and filarial cases has to be done.

Prevalence of arbo-viruses in India with reference to their genomics, meta-genomics 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. Also, metagenomic studies would yield information on the species invasion etc. which cause outbreaks of arbo-viral diseases hitherto unreported. 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 increase of the 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. The impact of this mutation on the protein conformation was studied using molecular dynamics simulations and the variations in backbone of the conformation was observed in the mutant envelop protein. Further, a comparative molecular docking study was carried for the wild type and mutant envelop proteins using a cross reactive monoclonal antibody. The results revealed that the binding orientation of the antibody is absolutely variable when compare with wild type docking complex. These studies

Figure 1.39 eCRF – Case sheet for Patients with Filarial Manifestations

implicates that the A219T mutation is responsible for the changes in the envelop protein and its conformations which could lead to increased resistance of dengue virus due to mutations.

Prediction and evaluation of antigenic determinants on proteins of *Wuchereria bancrofti*

Elimination of LF by Post MDA monitoring until 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.

Potential epitope regions were predicted in the Cuticular Collagen (CC) protein of *W. bancrofti* using multiple servers and their structural stabilities were analysed using molecular dynamics simulations. These predicted epitope models were located on the CC protein model to find out whether they are confined to the surface of the structure which will ensure the availability of the epitopes for potential immune interactions. By virtue of these analyses, the predicted epitopes were synthesised and in vitro evaluation was carried out.

The peptides were solubilised in TFA and PBS and the immunogenicity of each peptide was tested at concentrations ranging from 0.1–5.0 mg against known MF positive and negative sera by ELISA. Among the four peptides, CCEP2 and CCEP4 peptides showed higher immunogenicity at a concentration of 3.0 mg. In order to increase the specificity of the assay, the secondary antibody- peroxidase labelled anti-human IgG (IgG4) was used in place of anti-human IgG (whole molecule) in the assay protocol. The immunogenicity obtained for CCEEP2 and CCEP4 were 0.309 (0.04) and 0.327 (0.05) respectively showing improved specificity.

In order to maximize the immunogenicity, peptides were tested in cocktail form at three concentrations (0.4, 0.2 &0.1 µg), with different combinations. Among these, the combination of CCP2 and CCP4 at 0.2 µg showed immunoreactivity in terms of OD value 0.864 (0.181). Based on the results, combinational polyclonal anti-peptide antibody (for CCP2 and CCP4) generation in rabbits was outsourced and the evaluation is in progress.

Unit 2

Human Resource Development

2.1 HIGHER EDUCATION

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

2.1.1. M.Sc. Public Health Entomology

M.Sc. Public Health Entomology course (affiliated to Pondicherry University) continued for the academic year 2016–18, with a batch of 12 students. All the students are provided with a monthly stipend (open candidates: Rs. 6000/- & in-service candidates: Rs. 3000/-).

Four students who have completed the course (Academic year: 2014–16) were selected on inter-se merit basis and awarded one year paid (Rs. 12,000 + HRA / month) Internship at the Centre.

As a part of the course curriculum, the students visited National Centre for Disease Control (NCDC), New Delhi; National Vector Borne Disease Control Programme (NVBDCP), New Delhi; National Institute of Malaria Research (NIMR), New Delhi; Rajendra Memorial Research Institute (RMRI), Patna and NCDC Regional Station, Bengaluru. During these visits, they got direct exposure to the functioning of various National Institutes engaged in addressing different vector borne diseases in the country. The students were offered field training through VCRC Field Stations located in Koraput, Odisha and Kottayam, Kerala on Malaria and Dengue / Chikungunya in the respective areas.

2.1.2. Ph.D. Programmes

Two candidates (Zoology Full time – 1 and Microbiology Part time – 1) were awarded with Ph.D. degree. Fifteen full time (Zoology – 9; Microbiology – 5; Chemistry – 1) candidates and one part time (Zoology) candidate continue to pursue their doctoral programme. One of our internal staff has joined the Ph.D. programme (Zoology – 1) as part time candidate, during the current year.

2.2 STAFF & STUDENTS' VISIT

Staff and students from different academic and public health Institutions visited (1 day) VCRC for orientation and exposure to the various ongoing programmes of the Centre, and the details thereof are given below:

S.No.	Name of the College / Institution	Course / Title	No. of Staff / Students
1	Dept. of Personnel & Administrative, Delhi	CSS Level - D Cadre Officials	21
2	Kings Institute of Preventive Medicine & Research, Guindy, Chennai	M.Sc. Microbiology	12
3	Indira Gandhi Medical College & Research Institute, Puducherry	MBBS	97
4	Kandarswamy's Kandar College, Vellore	M.Sc. Zoology	60
5	Kendriya Vidyalaya, JIPMER Campus, Puducherry	Class XI & XII students	55
6	Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry	B.Sc. Nursing DMPHW(F) & LHW students	90 40
7	Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry	MBBS	180
8	Man Power Health Department, Villupuram	Health Workers	25

2.3 TRAINING

2.3.1. Entomological Training

Medical, Paramedical and Research scholars from other Institutions were offered entomological training for varying periods and the details are as under:

S.No.	Participants particulars	No. of Trainees	Field of Training	Period
1	MD Students, Dept. of Community Medicine, JIPMER, Puducherry	6	Integrated Vector Management, Surveillance system for Malaria & Filariasis and GIS mapping of VBDs	2nd Mar 2016
2	M.Sc. Community Health Nursing, College of Nursing, JIPMER, Puducherry	1	Mosquito identification	25th – 29th Jul 2016
3	MD Students, Dept. of Community Medicine, Sri Manakula Vinayagar Medical College and Hospital, Puducherry	4	Methods of Vector Control	22nd Aug 2016
4	Research Scholar from St. Joseph's College, Tiruchirapalli	1	Mosquito identification	19th – 23rd Sep 2016
5	MD Student, Dept. of Community Medicine, PIMS	1	Methods of Vector Control	13th – 14th Oct 2016
6	MD Students, Dept. of Community Medicine, JIPMER, Puducherry	4	Methods of Vector Control	26th – 27th Oct 2016

2.3.2. Formal Training Programmes

Scientists and Technical Staff from other National Institutes were given training on the following specialized fields:

S.No.	Participants particulars	No. of Trainees	Field of Training	Period
1	Entomologists from the Institute of Vector Control and Zoonoses, Hosur	2	Evaluation of Insecticide Impregnated papers	8th – 10th Feb 2016
2	Scientists from Centre for Research in Medical Entomology, Madurai	3	Rodent trapping, Collection of mites from the rodents, processing of the mite samples, mounting and identification of mites	1st – 3rd Mar 2016
3	Technical personnel from National Institute of Malaria Research, Chennai	3	Morphological identification of *Ae. albopictus* subgroup species	7th – 8th Jun 2016
4	Technical personnel from National Centre for Disease Control, New Delhi	7	Molecular methods of detecting Zika virus in *Aedes* mosquitoes	3rd – 7th Oct 2016

2.3.3. Sponsored Training Programme

National

A training programme for Biologists / Entomologists funded by NVBDCP, Delhi was organized at VCRC, Puducherry from 15th to 27th February 2016. A total of 25 trainees from 10 states have participated in the training programme. Senior Officials from the State Health Departments, Tamil Nadu and Experts from the NVBDCP, Delhi and VCRC, Puducherry have served as Resource persons. The training programme comprised of interactive lectures and laboratory demonstrations. Field visits to endemic areas were arranged to carry out situation analysis of VBDs, to understand the problems and constraints associated with control of VBDs and formulating suitable vector control strategies.

2.3.4. International

2.3.4.1. WHO Training Programme

World Health Organization (WHO) has sponsored Health Officials from Medical Research Institute, Colombo, Sri Lanka for entomology training at the Centre

S.No.	Participants particulars	No. of Trainees	Field of Training	Period
1	Health Officials from Medical Research Institute, Colombo, Sri Lanka	3	Entomology	12th – 18th Dec 2016

2.3.4.2. Foreign University Students' Project work

Students from foreign universities visited the Centre under the academic linkage programme for short term Project work and the details are given below:

S.No.	Name of the Student	Institution	Period (weeks)	Subject/Title
1	Ms. Hannah Elizabeth Cummins (Post Graduate)	Department of International Health, Georgetown University, Washington D.C, USA	11	Entomological risk characterization of scrub typhus in Puducherry, India
2	Ms. Eleanor Nevin Field (Under Graduate)	Department of International Health, Georgetown University, Washington D.C, USA	11	Entomological Risk Characterization of Dengue Transmission in Puducherry, India
3	Ms. Megan Marie Bristow	St. Olaf College, USA	6	Isolation and molecular detection of Bti from soil
4	Ms. Auste Joana Eigirdas	St. Olaf College, USA	6	Geo-statistical analysis of LF prevalence in India

2.4 NATIONAL SCIENCE DAY

National Science Day was celebrated on 8th April, 2016 in T.R. Rao Auditorium, VCRC. Dr. A. Chella Perumal, Professor & Head, Dept. of Anthropology, Pondicherry University delivered a talk on 'The Anthropology of vector-borne diseases'. Competitions were conducted for the Ph.D. Scholars and M.Sc. students in Essay writing, Elocution, Pencil Sketch, Chess & Carom. Prizes were distributed to the winners.

2.5 SWACHH PUDUCHERRY

As a part of Swachh Puducherry, launched by Hon'ble Lt. Governor of Puducherry, our PG students (M.Sc. PHE) and Ph.D. Scholars participated in cleaning campaign in Indira Nagar - Medical complex, Police quarters and Govt. Primary school premises on 20th and 21st October 2016. During this campaign, collection, segregation & removal of solid wastes were carried out.

Unit 3
Service and Supplies

3.1 EPIDEMIC INVESTIGATIONS

3.1.1. Entomological investigation of suspected JE/AES outbreak in Kotpad CHC, Koraput district, Odisha State

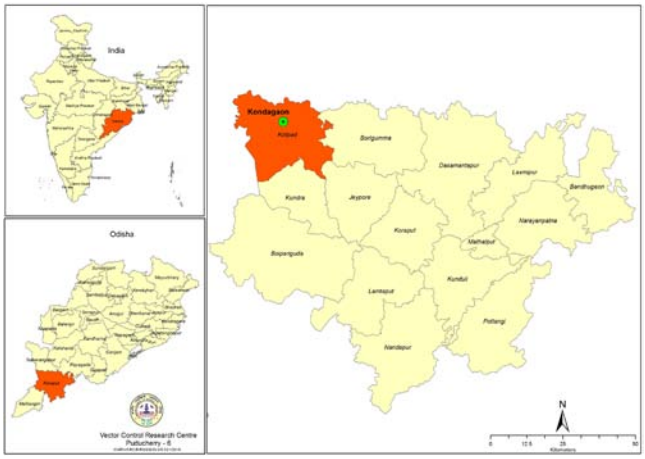
Sep 2016 – Oct 2016

Sahu SS, Dash S, Sonia T, Rao SP, Muthukumaravel S, Gunasekaran K, Jambulingam P

There was a report of two deaths due to suspected Japanese encephalitis (JE) occurred between 31/08/16 to 04/09/16 in Chitra Sevashram School, Chitra village, Kotpad CHC, Koraput District. The two deceased were from Kondagaon village of the same CHC. On the request of the Directorate of Health Services, Govt. of Odisha, the VCRC team headed by Dr. S.S. Sahu, Scientist E, along with Dr. K. P. Behera, DMO Koraput, visited Kondagaon and also the adjoining Banuaguda village for an investigation that included monitoring of abundance (density), human blood index, survival and dusk index of the known JE vectors.

The entomological survey conducted in the two villages yielded a total of 862 adult mosquitoes of 12 specie, 7 *Culex* and 5 *Anopheles* species. The per man-hour density (PMD) of *Cx. tritaeniorhynchus* was 36.9 and of *Cx. vishnui* was 25.4. While none of the 18 blood meals of *Cx. tritaeniorhynchus* tested for source of feeding was positive for human, the human blood index (HBI) of *Cx. vishnui* was 0.01 (n=85). With the prevailing entomological risk factors and detection of one IgM positive for JEV of the two serum samples (collected from the two deceased children), the district health department started implementing vector control intervention measures in the villages and the VCRC evaluated their impact on vector density. One day after indoor residual spraying with alphacypermethrin WP 5% (@ 25mg/m^2) and first round of fogging with 5% malathion, the PMD of *Cx. tritaeniorhynchus* was 28.8 and of *Cx. vishnui* was 39.5. Since, there was no marked reduction in the density of the JE vectors, second round of fogging was done after four days. Consequently, the PMD of the two vector species was reduced to 8.1 and 20.4, respectively. Further reduction in the vector density to 3.8 and 10.3, respectively was observed after third round of fogging. The dusk index of *Cx. vishnui* was also reduced from 13.8 to 2.1 after the intervention measures in the villages (Figure 3.1).

There were no further reports of fever cases or deaths in the villages. Extensive paddy cultivation with intermittent rainfall favoured high density of the JE vectors and this coupled with availability of reservoir and amplifying hosts and human feeding tendency of the vectors contributed to maintenance of entomological risk for JE transmission. The vector control intervention measures in the affected villages reduced the risk factors considerably.



Map showing the affected village in Kotpad CHC, Koraput district, Odisha State

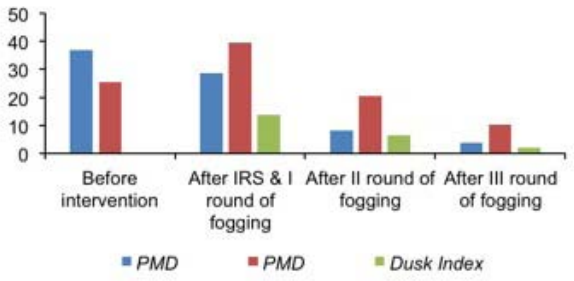


Figure 3.1 Impact of vector control intervention on density and dusk index of JE vectors

3.1.2. Entomological investigation of suspected JE/AES outbreak in Malkangiri District, Odisha State from 09-09-2016 to 14-09-2016

Malkangiri district of Odisha State recorded yet another outbreak of suspected JE/AES with admission of 424 cases with fever in the district headquarters hospital during the period from 9th September to 25th October 2016. This time, the outbreak occurred in wider geographical area as the suspected JE/AES cases were reported from 116 villages spread in seven Blocks and one municipality of the district. Serum samples of all the patients who got admitted in the hospital were

tested for JE IgM (ELISA) and of them 135 samples were found to be JE IgM positive. Of the remaining, 282 were reported to be AES cases. There were 76 deaths and among them deaths due to JE were 28 and the remaining 48 were diagnosed as non-JE/AES. Serum samples of 171 pig were subjected to ELISA and 91 were found positive for JE IgM.

The VCRC team visited the affected villages of the district and carried out entomological survey to identify the species prevalence, study the abundance of the recognized JE vector species, detect JE virus in the vector mosquitoes and to suggest appropriate vector control measures. *Culex vishnui* sub group of mosquitoes were more common in the affected villages. The per man hour density (PMD) and the dusk index (DI) of the JE vectors (*Cx. tritaeniorhynchus*, *Cx. vishnui*) ranged from 56.5 to 118.0 and 19.4 to 47.8 in different villages. A total of 259 blood meals of six species (*Cx. bitaeniorhynchus*=58, *Cx. fuscocephalus*=11, *Cx. gelidus*=5, *Cx. tritaeniorhynchus*=57, *Cx. vishnui*=70 and *Cx. whitmorei*=48) were analyzed for the source of feeding and one blood meal each of *Cx. vishnui* and *Cx. whitmorei* was positive for human with a HBI of 0.01 and 0.02, respectively. RT PCR assay of 651 vector mosquitoes in 58 pools detected one pool (25 mosquitoes) of *Cx. vishnui* mosquito positive for JE virus. These findings confirmed JE transmission in the affected area.

Since the vector density in the affected villages was high and the suspected JE/AES cases continued to occur, vector control intervention measures were implemented by the District Health Dept. with the technical support of VCRC. Six rounds of thermal fogging was done using malathion 5% cyphenothrin 5% covering 429 villages in the seven blocks. The VCRC evaluated the impact of the intervention measure. After the fogging operation, there was a reduction in the PMD and DI of the vector species. During the investigation, the VCRC team also trained five personnel of the State Health Dept. on entomological aspects of JE transmission including identification of JE vector species and the entomological parameters to be monitored during an outbreak.

3.2 FACILITIES

3.2.1. Laboratory animal facility

The Centres' laboratory animal facility has breeding colonies of animals such as BALB/c mice (*Mus musculus*), Mongolian gerbils (*Meriones unguiculatus*), and multimammate rats (*Mastomys coucha*). The facility is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests, Government of India. The animals maintained are being supplied for the Centres' own research projects approved by the Institutional Animal Ethics Committee (IAEC). During the reporting period, the IAEC meeting was conducted on 25th February 2016. Progress of one ongoing project and one completed project was reviewed by the committee. Apart from these, three new projects were approved for screening of wild rodents for detection of Scrub typhus and KFD pathogens. Health status of the animals maintained in the animal house facility is being monitored by a visiting veterinary doctor.

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

The sub-periodic strain of *Brugia malayi* filarial parasite is maintained in two animal models, viz., Mongolian gerbil (*Meriones unguiculatus*) and multimammate rat (*Mastomys coucha*). Mosquito stages of the parasite (L1 to L3) are developed in *Aedes aegypti* (Liverpool strain) by feeding them with microfilarae positive blood samples and the infective larvae (L3) obtained are inoculated to the animal models for development and patent infection. Presently, there are 14 multimammate rats and 9 mongolian gerbils inoculated with infective larvae of the parasite and out of these, 8 multimammate rats and 7 mongolian gerbils are with patent infection harbouring adult filarial parasite. Adults and mf collected from these animals are used for immunological, diagnostic and drug development studies. (Animal ethics committee approval).

3.2.3. Filaria Clinic

VCRC filarial clinic provides diagnostic, therapeutic and morbidity management services to lymphatic filariasis patients reporting from Pondicherry and field practice areas of Tamil Nadu. The numbers of patients who have attended the clinic is given in Table 3.1. Forty sixe lymphoedema patients visited first time which includes patients referred from territory care hospitals like JIPMER and IGGH

Pondicherry. Twenty seven 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 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 like government hospital Puducherry, JIPMER and one case to Karaikal Government hospital.

TABLE 3.1

Number of patients who availed the clinical services during Jan–Oct 2016.

Clinical Diagnosis	Number of Patients		
	First Visit	Repeat Visits	Total Visits
LE Grade-I	0	2	2
LE Grade-II	25	204	229
LE Grade-III	11	209	220
LE Grade-IV	10	165	175
Others*	26	18	44
Total	72	598	670
ADLA#	9	18	27

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

3.2.4. Rearing and colonization of mosquitoes

Cyclic colonies of the following four species of mosquitoes are being maintained in the Rearing and Colonization laboratory. Immature adult mosquito specimens 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 Dy. Director of Health Services Office, Cuddalore 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 spledens*

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

- *Gambusia affinis*
- *Poecilia reticulate*

Supplies from Rearing & Colonization Division to other laboratories of VCRC and outside institution are given in Table 3.2.

3.2.5. Mosquito museum

A mosquito museum was established in VCRC in the year 2005 with the aim to collection of mosquito species prevalent in India and would serve as reference material for taxonomic studies and training in mosquito identification. Updating

TABLE 3.2

Supplies from Rearing & Colonization Division to different laboratories during Jan–Oct 2016

Species	Internal (within VCRC)					External*	Total
	Vector Biology & Control	Microbiology, Immunology & Bioinformatics	Human Resource Development	Chemistry	Vector Ecology & Surveillance	Dy. Director of Health Services Office, Cuddalore	
Culex quinquefasciatus							
Immature stages	1500	121550	43050	33100	900		**200100**
Adults	–	250	1550	500	100		**2400**
Anopheles stephensi							
Immature stages	14400	77200	300	4100	800		**96800**
Adults	3350	300	1750	–	200		**5600**
Aedes aegypti							
Immature stages	–	97800	4700	22400	1800	1200	**127900**
Adults	–	–	700	6000	600	300	**7600**

*Supplies were provided for Exhibition purpose

the museum specimens from mangrove forests including Andaman & Nicobar Islands and different ecosystems in the geographical regions in India between 1995 and 2015 were deposited. At present there are 40,349 adult specimens, of which 30,870 were individually pinned and the remaining preserved in stock vials. It also includes 3688 male/female genitalia, 5868 larvae, 6283 larval skin and 3766 pupal skin. Representative specimens of different species are available from 18 states and 3 union territories of India. The updated information was published periodically in the "Journal of American mosquito control association".

List of new country records: 26 species comprising 8 genera namely, *Aedes (Diceromyia) franciscoi, Aedes (Lorrainae) amesii, Aedes (Lor.) fumidus, Ayurakitia peytoni, Armigeres (Armegeres) pallithorax, Coquillittidia (Coquillittidia) richiardii, Culex (Culex) pipens, Culex (Cux.) luzonensis, Culex (Culiciomyia) spathifurca, Culex (Cui.) thurmanorum, Culex (Lopocearaomyia) aculeatus, Culex (Lop.) cubitatus, Culex (Lop.) demissus, Culex (Lop.) gracicornis, Culex (Lop.) inculus, Culex (Lop.) paraculeatus, Culex (Lop.) pilifemuoralis, Culex (Lop.) wardi, Culex (Lop.) wilfredi, Heizmannia (Heizmannia) chengi, Ochlerotatus (Finlaya) feegradei, Ochlerotatus (Fin.) flavipennis, Ochlerotatus caspius, Verrallina (Harbachius) consonensis, Verrallina (Har.) lankensis, Uranotaenia (Uranotaenia) rutherfordi.*

List of new species: *Culex (Lophoceraomyia) singhbhumensis, Ochlerotatus (Finlaya) cherrapungiensis.*

Mosquito species collected: 292/402 number, about 74% of mosquito is known in India.

Identification service: Specimens brought by public health personnel and Ph.D scholars from different colleges/University.

Unit 4
Publications

1. Gunasekaran K, Sahu SS, Vijayakumar T, Subramanian S, Yadav RS, Pigeon O and Jambulingam P. An experimental hut evaluation of Olyset Plus, a long-lasting insecticidal net treated with a mixture of permethrin and piperonyl butoxide, against *Anopheles fluviatilis* in Odisha State, India. *Malaria Journal* 2016, 15(1):375.
2. Jambulingam P, Subramanian S, de Vlas SJ, Vinubala C and Stolk WA. Mathematical modelling of lymphatic filariasis elimination programmes in India: required duration of mass drug administration and post-treatment level of infection indicators. *Parasite and Vectors* 2016, 9:501.
3. 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* 2016, 34(8):1736–1750.
4. Natarajan R, Rajavel AR and Jambulingam P. Description of a new species of the genus *Hulecoeteomyia (Diptera: Culicidae)* from Meghalaya, India. *Zootaxa* 2016, 4137(3): 330–338.
5. 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 Zoonotic Diseases* 2016, 16(2):117–123.
6. Saratha R and Nisha M. Development of a mosquito attractant blend of small molecules against host-seeking *Aedes aegypti*. *Parasitology Research* 2016, 115(4): 1529–1536.
7. Srinivasan R, Pradeep Kumar N and Jambulingam P. Detection of natural infection of *Leishmania donovani (Kinetoplastida: Trypanosomatidae)* in *Phlebotomus argentipes (Diptera: Psychodidae)* from a forest ecosystem in the Western Ghats, India, endemic for cutaneous leishmaniasis. *Acta Tropica* 2016, 156:95–99.
8. Vasuki V, Subramanian S, Sadanandane C, Jambulingam P and Abdul Khader MSM. Molecular xenomonitoring of *Wuchereria bancrofti* in *Culex quinquefaciatus* mosquitoes from an endemic area: Comparison of two DNA extraction methods for real time PCR assay. *Journal of Vector Borne Diseases* 2016, 53(1):77–80.
9. Yogeswari S and Srinivasan R. A Note on Variations in Morphological Features of the Phlebotomine Sand Fly *Sergentomyia bailyi* (*Diptera: Psychodidae*) in a Population from Pondicherry UT, India. *Journal of Medical Entomology* 2016, 53(3): 712–716.
10. Athisaya Mary K, Krishnamoorthy K and Hoti SL. Scope of detectability of circulating antigens of human lymphatic filarial parasite *Wuchereria bancrofti* with smaller amount of serum by Og4C3 assay: its application in lymphatic filariasis elimination programme. Journal of Parasitic Diseases 2016, 40(4): 1622–1666.
11. Chauhan N and Hoti SL. Role of cysteine-58 and cysteine-95 residues in the thiol di-sulfide oxidoreductase activity of Macrophage Migration Inhibitory Factor-2 of *Wuchereria bancrofti*. Acta Tropica 2016, 153:14–20.
12. Chauhan N and Hoti SL. An alternative strategy to generate coding sequence of macrophage migration inhibitory factor-2 of *Wuchereria bancrofti*. Indian Journal of Medical Research 2016, 143(2):232–237.
13. Das BP, Deobhankar K, Pohekar KN, Marathe R, Husain SA and Jambulingam P. Laboratory Bioassay of *Chilodonella uncinata*, an Entomopathogenic Protozoan, against Mosquito Larvae. Journal of Mosquito Research 2016, 6(10):1–10.
14. Jeelani S and Sabesan S. Dengue vector abundance and diversity of breeding habitats in Puducherry, South India. Tropical Biomedicine 2016, 33(1):71–77.
15. Sadanandane C, Elango A, Paily KP, Agatheswaran S and Jambulingam P. Abundance & distribution of trombiculid mites & *Orientia tsutsugamushi*, the vectors & pathogen of scrub typhus in rodents & shrews collected from Puducherry & Tamil Nadu, India. Indian Journal of Medical Research 2016, 144(6):893–900.
16. Sahu SS, Rao SP and Dash S. Performance of Accredited Social Health Activists (ASHAs) in diagnosis and treatment of Malaria in Eight Falciparum Endemic Tribal Districts of Southern Odisha, India. The Journal of Communicable Diseases 2016, 48(2):12–19.
17. Michael AI, Wilma/Wilhelmina AS, Morgan ES, Swaminathan S, Brajendra KS, Gary JW, Edwin M and T Deirde Hollingsworth. Effectiveness of a triple-drug regimen for

global elimination of lymphatic filariasis: a modelling study. *Lancet Infect Dis* 2016, S1473-3099(16)30467-4.

18. Pradeep Kumar N. Featured article - World Biomedical Frontiers - biomedfrontiers.org/inf-2016-6-4/ (Acta Trop 2016 Apr, 156: 95–99 - Abstract with Supplement)
19. Pradeep Kumar N, Rajavel AR and Jambulingam P. Development of a PCR methodology to distinguish species members of *Culex vishnui* subgroup *(Diptera: Culicidae)*, based on DNA Barcodes. *Insect Science* 2016, doi: 10.1111/1744-7917.12344.
20. Sadanandane C, Elango A, Noonu M, Sasidharan PV, Raju KHK and Jambulingam, P. An outbreak of Kyasanur forest disease in the Wayanad and Malappuram districts of Kerala, India. Ticks and Tick-Borne Diseases, doi.org/10.1016/j.ttbdis.2016.09.010.

Book Chapter Published

1. Nanda Kumar Y. "Molecular Modelling, Dynamics, and Docking of Membrane Proteins: Still a Challenge." Applied Case Studies and Solutions in Molecular Docking-Based Drug Design. *IGI Global*, 2016. 186–208. doi:10.4018/978-1-5225-0362-0.ch007.

In Press

1. Sadanandane C, Elango A, Paily KP, Patricia Anitha K, Agatheswaran S and Jambulingam P. (2016) Field evaluation of the bio-larvicide, spinosad 20% EC in comparison to its 12% SC formulation against *Culex quinquefasciatus*, the vector of bancroftian filariasisin India. *Indian Journal of Medical Research* (2016).
2. Sadanandane C, Gunasekaran K, Boopathi doss PS and Jambulingam P. Prevalence and abundance of Trombiculid mites and *Orientia tsutsugamushi*, the vectors and pathogen of scrub typhus in rodents and shrews collected from areas reported for human cases of scrub typhus in Pondicherry, India. *Indian Journal of Medical Research* (2016).
3. Tamilselvan S, Jambulingam P and Manonmani AM. Fly ash based water dispersible powder formulation of *Bacillus thuringiensis* var. i*sraelensis* - development and laboratory evaluation against mosquitoes. *Indian Journal of Medical Research* (2016).

Patent

1. Nisha Mathew, Paily KP. *In vitro* ADME studies of antifilarial substituted naphthoquinones, 2016.

Unit 5
Meetings / Seminars / Symposia / Confernces / Workshops / Guest Lectures Delivered

Date	Particulars	Scientist
6–9 Jan	Attended the demonstration of the operation of GC/MS at NIMR field station at Haridwar	Dr. Nisha Mathew
7 Jan	As Chief guest delivered a lecture on “Neoteric tactics in Microbial Era “at the National Seminar organized by Shanmuga Industries Arts & Science, Tiruvannamalai.	Dr. A.M. Manonmani
9 Jan	Advocacy workshop on “Mosquitogenic conditions prevailing in Puducherry and role of mosquitoes in transmitting dengue and other VBDs” for self-help groups & Sanitary workers organized at Kamban Kalai Arangam, Puducherry by Dept. of Health and Family Welfare, Govt. of Puducherry	Dr. K. Gunasekaran
9 Jan	Attended a district level malaria review and special drive for special IRS planning meeting at DHH Koraput under the chairmanship of the CDMO, Koraput and all MTSs Koraput, DMO and VBD consultant, Koraput	Dr. S.S. Sahu
20 Jan	Participant in the College Nodal Officer’s meet held at Pondicherry University.	Dr. A.M. Manonmani
25 Jan	Attended Consultation meeting on the use of revolutionary CRISPR/Cas9 technology for solving health challenges in India as well as other novel initiatives such as exploring the use of genetically modified mosquitoes for malaria control organized by TATA Trusts, Mumbai	Dr. P. Jambulingam
27 Jan	As member, Special Board of studies for Ph.D programmes in Medical microbiology & Medical Entomology, RMRC, Port Blair framed the syllabi for these courses	Dr. A.M. Manonmani
2 Feb	Attended and delivered an invited lecture on “Exploring the potential of medicinal plants for the control of Vector Borne Diseases“ at the National Seminar on “Medicinal Plant Bio-diversity & Local Health care” organized by Sri Parasakthi College, Courtallam, Tamil Nadu	Dr. I. Geetha
4 Feb	Attended a meeting on development of pre-monsoon strategies for control of arbo-viral diseases in Kerala at Directorate of Health Services, Govt. of Kerala	Dr. N. Pradeep Kumar
10–11 Feb	Attended National Meeting for launching “National Framework for Malaria Elimination (NFME) in India (2016–2030) organized by Dte. of National Vector Borne Disease Control Programme with the support of WHO-SEARO and WHO Country Office at Regency Ballroom, Hyatt Regency, in New Delhi	Dr. P. Jambulingam
15–16 Feb	TDR/WHO supported workshop on courses on Vectors and Vector-borne diseases, Libson, Portugal	Dr. P. Jambulingam
15–19 Feb	Undergone training on “Zika virus diagnosis” at National Institute of Virology, Pune	T. Sankari S. Muthukumaravel
15–27 Feb	Participated as resource person in the Biologists Training Workshop conducted at VCRC	Dr. K.P. Paily
15–27 Feb	Biologist training jointly organized by VCRC and NVBDCP at VCRC Pondicherry	Dr. K. Gunasekaran Dr. S. Subramanian
17–18 Feb	Attended and delivered an invited lecture on “Need for Research Based Curriculum in PG Microbiology Courses” in National Workshop on Curriculum designing in Microbiology organized by St. Joseph’s College, Cuddalore, Tamil Nadu	Dr. I. Geetha
22–23 Feb	Participated the ‘Clinical experts meeting on Lymphatic filariasis’ organized by DNDi in collaboration at ICMR, New Delhi	Dr. Nisha Mathew
24 Feb	Attended a meeting at Health Secretariat, Bhubaneswar on ‘’Prevalence and distribution of haemoglobinopathies (Sickle cell and Thalassemia) and G6PD deficiency in relation to malaria among tribal groups in Odisha State, India” under the chairmanship of Health Secretary, Govt. of Odisha organised by Health Department, Govt. of Odisha	Dr. S.S. Sahu
26 Feb	Participated in the meeting of state and District entomologists and Public Health personnel to organize the ZIKA vector surveillance program in Kerala at Directorate of Health Services, Govt. of Kerala	Dr. N. Pradeep Kumar
29 Feb	Meeting of the Institutional Human Ethics Review Board (IHERB), Central University of Tamil Nadu (CUTN) at the University Hall, Thiruvarur	Dr. K. Gunasekaran
1 Mar	Attended Meeting of Technical Advisory Committee(TAC) of NVBDCP, DGHS, 4th floor, Nirman Bhawan, New Delhi	Dr. P. Jambulingam
1 Mar	Guest lecture on Vector Control Methods and Insecticide Resistance to the M.Sc. Life Science Students, Central University of Tamil Nadu (CUTN), Thiruvarur	Dr. K. Gunasekaran
2 Mar	Attended Expert group meeting to discuss the new as well as existing alternate strategies for vector control for *Aedes* mosquitoes at ICMR, Hqrs	Dr. P. Jambulingam
2 Mar	Attended a Research and ethics meeting at the chamber of the CDMO Malkangiri to discuss about the project on ‘’Prevalence and distribution of haemoglobinopathies (Sickle cell and Thalassemia) and G6PD deficiency in relation to malaria among tribal groups in Odisha State, India” with the CDMO, DMO and VBDC Malkangiri	Dr. S.S. Sahu

Date	Particulars	Scientist
3–4 Mar	National Seminar on Faunal Diversity of Eastern Ghats and Western Ghats held at Zoological Survey of India, Chennai Dr. V. Vasuki: Oral presentation given on "Diversity of mosquitoes in the selected areas of Eastern Ghats: a review" Dr. R. Srinivasan: Presented a paper "Sandfly fauna (Diptera: Psychodidae) of southernmost part of the Western Ghats" Dr. C. Sadanandane: Presented a paper on "Prevalence and distribution of Ixodid ticks in the forest fringes of Western Ghats"	Dr. V. Vasuki Dr. R. Srinivasan Dr. C. Sadanandane
22 Mar	Collaborative meeting to strengthen malaria control programme in Odisha organized at RMRC, Bhubaneswar	Dr. P. Jambulingam Dr. K. Gunasekaran Dr. S.S. Sahu
23 Mar – 17 Sep	Participated as external Scientist member in the Institutional Animal Ethics Committee meetings of Pondicherry University	Dr. K.P. Paily
1 Apr	Invited lecture delivered on "Identification of pathogens in vectors" to 4thyr. Integrated M.Sc. students at Central University, Thiruvarur	Dr. V. Vasuki
7 Apr	Attended the Mass spectrometer demonstration at Biochemistry Department, JIPMER	Dr. Nisha Mathew
8 Apr	Attended Awareness-cum-training workshop on "Bio-Medical waste and e-waste Management" at Dr. A. P. J. Abdul Kalam Science Centre, Pondicherry-7, organized by Puducherry Pollution Control Committee, Dept. Science & Technology & Environment, Govt. of Puducherry	Dr. V. Vasuki
11 Apr	Attended a Zonal level malaria review and first round of IRS planning meeting at DHH Rayagada with DMOs and VBDCs of 11 districts of southern Odisha under the chairmanship of the Joint Director, Malaria Bhubaneswar, Govt. of Odisha	Dr. S.S. Sahu
13 Apr	Delivered guest lecture on 'Diagnosis of Vector Borne Diseases' for Life Science Post graduate students of Central University of Tamil Nadu, Thiruvarur	Dr. K.P. Paily
22 Apr	Attended a meeting at Health Secretariat, Bhubaneswar on ''Prevalence and distribution of G6PD deficiency in relation to malaria among tribal groups in Odisha State, India" under the chairmanship of Health Secretary, Govt. of Odisha organised by Health Department, Govt. of Odisha	Dr. S.S. Sahu
25 Apr	Attended a district level meeting for observation of ''World Malaria Day" at seminar Hall of CDMO Koraput under the chairmanship of the CDMO, Koraput	Dr. S.S. Sahu
26 Apr	Participated in the Meeting held at ICMR, New Delhi for finalizing the core research areas of VCRC for the ICMR Strategic Plan document 2017–2022	Dr. A.M. Manonmani
27–28 Apr	Attended Meeting of Regional Dengue Task Force, Male, Maldives	Dr. P. Jambulingam
10 May	Participated in a brain storming one day workshop on the basic concepts of climate change and its impacts on health for the officials of Health Services and other associated institutions at Vivanta by Taj, Trivandrum	Dr. N. Pradeep Kumar
20 May	Participated in the Meeting held at ICMR, New Delhi for reviewing ICMR technologies under translational research and presented the update on technologies related tothrombinase and Cyclosporin A	Dr. A.M. Manonmani
24 May	Participated in the Seminar on Gene Drive Technology and its applications organized by ICGEB/NII/NIPGR at National Institute of Immunology, Delhi	Dr. N. Pradeep Kumar
26 May	Participated in the meeting at District Collectorate at Kottayam on tackling dengue outbreak situation in Kottayam.	Dr. N. Pradeep Kumar
28 May – 4 Jun	Attended ''QUALITY MANAGEMENT SYSTEM WORKSHOP at Penang, Malaysia' organised by WHO	Dr. S.S. Sahu
30 May – 3 Jun	Attended "Quality Management System Workshop" sponsored by the WHO, held at Penang, Malaysia	Dr. K. Gunasekaran Dr. R. Srinivasan
10 Jun	XI joint annual conference of Indian Society for Malaria and other communicable diseases & Indian Association of epidemiologists "Public Health in Digital India and Swachh Bharat"	Dr. P. Jambulingam
10 Jun	Attended a meeting on ZIKA Surveillance project progress in Kerala state at DHS, Kerala, Trivandrum	Dr. N. Pradeep Kumar
16 Jun	Delivered a lecture on ''Ecology and Bionomics of malaria vectors in India'' in the Entomological Training Workshop organized by Department of Community Medicine, Medical College Trivandrum to Post Graduate students of Community Medicine of different Medical Colleges of four southern States of India.	Dr. S.S. Sahu
17 Jun	Attended Meeting to discuss the alternative strategies for vector control at ICMR Hqrs., New Delhi	Dr. P. Jambulingam

Date	Particulars	Scientist
27–28 Jun	GIS Consortium - Vision Development Workshop, Sri Balaji Vidyapeeth, MGMC&RI, Puducherry	Dr. P. Jambulingam
1 Jul	National Consultation on Acute Encephalitis Syndrome (AES) at ICMR Hqrs, New Delhi	Dr. P. Jambulingam
4 Jul – 8 Aug	WHO Short-Term Consultant on Malaria Entomology at DPR of Korea: Provided on-site training to the Central level entomologists	Dr. K. Gunasekaran
8 Jul	Attended Awareness-cum-training workshop on "Bio-Medical waste and e-waste Management, at Dr. A. P. J. Abdul Kalam Science Centre, Pondicherry-7, organized by Puducherry Pollution Control Committee, Dept Science & Technology & Environment, Govt. of Puducherry	Dr. V. Vasuki
22 Jul	Attended a district level malaria review meeting at CDMO Conference Hall under the chair-manship of the CDMO, Koraput and all MTSs Koraput, DMO and VBD consultant, Koraput participated in the meeting	Dr. S.S. Sahu
26 Jul	Participated in the workshop on 'Human Bioethics" organized by Division of Research & Department of Clinical Pharmacology, JIPMER	Dr. Nisha Mathew
12 Aug	Selection of candidates for the post of Scientist C & B held at Yadgir district for IDA study at Yadgir, Karnataka	Dr. P. Jambulingam
12 Aug	Attended the 'Meeting of the Board of studies in Chemical Oceanography" in the syndicate room of Cochin University of Science & Technology, Cochin, Kerala.	Dr. Nisha Mathew
19–20 Aug	Expert Consultation on Kala-azar vector control in South East Region supported by SEARO, WHO organized by Central University of Tamil Nadu, Thiruvarur, Alwarpet, Chennai	Dr. P. Jambulingam
29 Aug	Participated in the ICMR sponsored National conference on "Emerging trends in Target based drug discovery" as a resource person and gave a lecture on 'Antifilarial Drug Development – a target based approach'	Dr. Nisha Mathew
30 Aug	Participant in the meeting of College Principals held at Pondicherry University	Dr. A.M. Manonmani
1 Sep	Expert Review Group Meeting for evaluation of Public Health Pesticides at ICMR	Dr. K. Gunasekaran
1–3 Sep	Attended the "India-Africa Health Sciences Meet" in Vigyan Bhawan, New Delhi and displayed the technology "*Bacillus thuringiensis* var. *israelensis* (VCRC B17) (MTCC 5596), a mosquitocidal biopesticide" at the 'Exposition of innovative technologies and programs' held during this meet	Dr. A.M. Manonmani
1–3 Sep	Capacity building activity for disease burden estimation, an interactive workshop on Global Burden of Disease (GBD) techniques organized by ICMR at NIOP, New Delhi	Mrs. A. Srividya
2 Sep	Attended Joint ICMR-ICAR Meeting on Task Force in Rickettsial Infections at ICMR Hqrs. New Delhi	Dr. P. Jambulingam
5–9 Sep	Burden of Disease (BOD) working group meetings held at PHFI, New Delhi	Mrs. A. Srividya
9 Sep	Attended a meeting with the CDMO and all district level Medical Officers regarding JE outbreak at Kotpad Block, Koraraput district at CDMO Koraput chamber under the chairmanship of the CDMO, Koraput	Dr. S.S. Sahu
13–14 Sep	Conference on Cities, Climate Forcing and Infectious Diseases organized by University of Chicago, New Delhi	Dr. P. Jambulingam
20–21 Sep	Attended 10th Meeting of the Global Collaboration for the Development of Pesticides for Public Health (GCDPP), WHO, Geneva, Switzerland	Dr. P. Jambulingam
23 Sep	Attended a meeting with the DMO and VBD consultant Malkangiri regarding JE outbreak at Kotpad Block, Koraraput at DMO chamber Malkangiri	Dr. S.S. Sahu
26–28 Sep	Eliminate Dengue Project, funded by ICMR at Monash University, Melbourne, Australia	Dr. P. Jambulingam
28 Sep – 1 Oct	Eliminate Dengue Project, Indonesia, funded by ICMR, Universities Gadjah Mada, Yogyakarta, Indonesia	Dr. P. Jambulingam
30 Sep	Participant in the meeting of College Principals regarding "Swaach Puducherry" held at Pondicherry University	Dr. V. Vasuki
1 Oct	Attended a district level malaria review and IRS planning meeting at residential chamber of the Collector and District Magistrate Koraput under the chairmanship of the Collector Koraput and all district level medical Officers, he CDMO Koraput, DMO and VBD consultant, Koraput participated in the meeting	Dr. S.S. Sahu
4 Oct	ICMR-WHO Regional Consultation on "National Ethical Guidelines for Biomedical and Health Research involving Human Participants, 2016, National Centre for Disease Informatics and Research (NCDIR), Bengaluru	Dr. P. Jambulingam
6–10 Oct	Attended district level meetings on control of JE outbreak at Malkangiri at to residential chamber of the Collector and District Magistrate Malkangiri under the chairmanship of the Collector Malkangiri; the state level health department officials and all the district level officers of Malkangiri district participated in the meetings	Dr. S.S. Sahu
12 Oct	Attended a district level preparedness meeting on prevention of JE at Koraput at Sadvabana Hall, DNK, Koraput under the chairmanship of the Health Secretary, Govt. of Odisha, BBSR; the Collector Koraput, and all the district level officers of Koraput district participated in the meeting	Dr. S.S. Sahu

Date	Particulars	Scientist
10–12 Nov	Annual meeting of the Coalition for Operation Research on Neglected Tropical Diseases (COR-NTD) programs to review the progress on modelling lymphatic filariasis held at Atlanta, Georgia, USA	Dr. S. Subramanian
14 Oct	Workshop on "Basic and Applied Bioinformatics for Clinical Genomics". Central Inter-Disciplinary Research Facility (CIDRF), Sri Balaji VIdyapeeth, Mahatma Gandhi Medical College, Puducherry. Lecture and hands on given: "Molecular dynamics of non-synonymous SNPs".	Dr. Y. Nanda Kumar
13–17 Nov	Annual meeting of the American Society for Tropical Medicine and Hygiene (ASTMH) held at Atlanta, Georgia, USA	Dr. S. Subramanian
17 Oct	Participant in the meeting of College Principals regarding "Swaach Puducherry" held at Pondicherry University	Dr. V. Vasuki
17–18 Oct	Sensitization Workshop on GLP, jointly organized by Department of Science and Technology National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA) at India Habitat Centre, New Delhi	Dr. K. Gunasekaran Dr. S.S. Sahu Dr. R. Srinivasan
19–21 Oct	Participated at `The Regional Consultation of WHO Collaborating Centres in South-East Asia Region held at Hotel Le Meridien, New Delhi wherein a poster describing the achievements of the institute and the invention on Bti along with pamphlets of the Bti product supplied by its licencees, various types of formulations and the booklet on `Achievements of VCRC' and `Brochure of M.Sc PHE course' were displayed at the market place set aside for ICMR	Dr. A.M. Manonmani
22 Oct	Attended CME on "Infectious disease update: Theme-Current management of bacterial infections" organized by JIPMER	Dr. I. Geetha, T. Sankari S. Muthukumaravel
26 Oct	Attended GBD India Vector Borne and Neglected Tropical Diseases Expert Group Meeting at ICMR Hqrs. New Delhi	Dr. P. Jambulingam
26–27 Oct	Attended a Brainstorming meeting ''Future scopes and Challenges on Research in Medical Entomology in India'' at CRME Madurai	Dr. S.S. Sahu
3–4 Nov	Expert Team meeting on JE/AES of Odisha at the National Health Mission (Govt. of Odisha) Office, Bhubaneswar	Dr. K. Gunasekaran
3–5 Nov	Attended Visceral Leishmaniasis Consortium meeting organised by LSHTM, New Delhi, India	Dr. P. Jambulingam Dr. R. Srinivasan
6 Nov	Participated as a member in the expert committee meeting of the Inter University Centre for Biomedical Research & Super Speciality Hospital (IUCBR & SSH), Kottayam held at the Secretariat, Trivandrum, for assessing and ranking prospective candidates for appointment as Senior Scientists.	Dr. N. Pradeep Kumar
7 Nov	Participant in the meeting of College Principals regarding "Swaach Puducherry" held at Pondicherry University	Dr. V. Vasuki
28–30 Nov	Workshop On The Development Of Signalling Pathway Networks And Analysis Of Transcriptomics And Proteomics Data". Rajiv Gandhi Centre for Biotechnology, Trivandrum	Dr. Y. Nanda Kumar
10 Dec	Second Meeting of Performance Evaluation Committee (PEC) held at ICMR, New Delhi	Dr. P. Jambulingam


VECTOR CONTROL RESEARCH CENTRE
PONDICHERRY
International Day of Yoga
21.06.2016

Unit 6
Celebrations

6.1 OFFICIAL LANGUAGE IMPLEMENTATION, 2016

राजभाषा (हिन्दी) वार्षिक रिपोर्ट – 2016

ईस वर्ष के दौरान केंद्र में राजभाषा कार्यान्वयन में प्रगति हुई है। जेसे की हिंदी में प्राप्त पत्रों को हिंदी में जवाब जारी और सभी पत्रसिर, साइन बोर्ड, नाम प्लेटें, दिशात्मक संकेत, रबर स्टांप, विजिटिंग कार्ड और उपस्थिति रजिस्टरों द्विभाषी हे । इसके अलावा केंद की दो कर्मचारियों (निजी सचिव और सदस्य सचिव) ने नगर राजभाषा कार्यान्वयन समिति (TOLIC) और जीपमार (JIPMER) द्वारा आयोजित दो दिवसीय राजभाषा कार्यशाला में भाग लिये। ईस वर्ष हिंदी शिक्षण योजना में दो कर्मचारियों ने प्रशिक्षण लिये हैं, और साल मे चार त्रैमासिक राजभाषा कार्यान्वयन समिति (VCRC OLIC) आयोजित की गई। तिमाही प्रगति रिपोर्ट आई. सी. एम. आर (ICMR), नई दिल्ली को भेजा गया था। नगर राजभाषा कार्यान्वयन समिति (TOLIC), जीपमार (JIPMER), पुधुच्चेरी और क्षेत्रीय कार्यालय, कोचीन को दो अर्ध - वार्षिक रिपोर्ट भेजा गया था। वर्ष के दौरान VCRC OLIC के सदस्य सचिव जीपमार (JIPMER), पुधुच्चेरी में नगर राजभाषा कार्यान्वयन समिति के बैठकों में भाग लिये। इसके अलावा कर्मचारियों ने नगर राजभाषा कार्यान्वयन समिति, जीपमार द्वारा आयोजित हिन्दी पखवाड़े उत्सव के अवसर पर आयोजित विभिन्न प्रतियोगिता में भाग लिये।

6.2 INTERNATIONAL WOMEN'S DAY, 2016

In view of the International Women's Day 2016, a celebration in the centre was observed on March 8th 2016 with Dr. R Murali, Dean of Mother Teresa Post graduate and Research Institute of Health Sciences as the Chief Guest. In connection with this event a health screening for women staff to detect early signs of cancer was jointly organised with the Department of Surgery, JIPMER, Puducherry. Various competitions were also held for the women staff and students of the centre and the winners were given prizes.

6.3 ACTION TAKEN REPORT ON THE CELEBRATION OF CONSTITUTION DAY AT VECTOR CONTROL RESEARCH CENTRE, PUDUCHERRY

With reference to the mail received on 25th November 2016 from MHRD, GOI, New Delhi, the constitution day was celebrated at our Centre on 26th November, 2016 at 11 am. The programme was headed by our Director Dr. P. Jambulingam and the Chief, HRD, Dr. A. M. Manonmani. All the M.Sc. Public Health Entomology students and Ph.D. scholars took part in the programme.

An introductory talk was given by our Chief, HRD Dr A. M. Manonmani. Her talk was revolving around the topic constitution day and the importance of constitution day. One of the M.Sc. Intern presented brief description about the eleven fundamental duties formulated on 26th November 1949. Finally, the pledge was taken by all Faculty, staff and students.

Banner displayed on Constitution day

Introductory talk on Constitution day by Dr. A. M. Manonmani

Talk on the eleven fundamental duties framed on Nov 26th 1949

Pledge taken by the faculty members, students and other staffs

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

Shri. G. Dhakshinamoorthy
Member
30, III Cross, Jhansi Nagar, Mudaliarpet,
Puducherry – 605 004

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
Director
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
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. (Mrs.) V. Vasuki
Member
Scientist 'D'
Vector Control Research Centre,
Indira Nagar, Puducherry – 605 006

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

Mrs. Vasumathi Nagarajan
Member
Account Officer
Vector Control Research Centre,
Indira Nagar, Puducherry – 605 006

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

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

Mr. 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.) K. Athisaya Mary	*Member*
Mr. S. Balasubramanian	*Member*
Dr. R. Natarajan	*Member*
Mr. G. Jeeva	*Member*
Mrs. G. Vijayalakshmi	*Member*
Mr. G. Prabhakaran	*Member*
Mr. R.S. Mariappan	*Member*
Mrs. A.V. Chandrakala	*Member*
Mr. B. Kumaresan	*Member*
Mr. P. Kumaran	*Member*
Mr. S. Maria Joseph	*Member*
Mr. N. Ramesh	*Member*
Mr. T. Mohanan	*Member*
Mr. A. Murugasamy	*Member*
Mr. Sundaresan	*Member*
Dr. R.L.J. De Britto	*Member Secretary*

Recruitment Cell

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. S. Subramanian	*Member*
Mrs. Vasumathi Nagarajan	*Member Secretary*

Electronic File Management System Committee

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

General Maintenance Committee

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. (Mrs.) V. Vasuki	*Member*
Dr. R. Srinivasan	*Member*
Mr. S. Balasubramanian	*Member Secretary*

Condemnation Committee

Prof. N. Sakthivel	*Chairman*
Prof. C.P. Prince	*External Member*
Dr. V. Vasuki	*Member*
Mrs. B. Parassacty	*Member*
Mrs. Vasumathi Nagarajan	*Member*
Dr. C. Sadanandane	*Member Secretary*

Environmental Safety Committee/Biosafety Committee

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

Purchase Committee

Dr. K.P. Paily	*Chairman*
Dr. S. Subramanian	*Member*
Dr. Nisha Mathew	*Member*
Dr. V. Vasuki	*Member*
Mr. 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*
Mr. 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. Kandasami	*Member Secretary*

Vehicle Maintenance Committee

Dr. S. Subramanian	*Chairman*
Dr. C. Sadanandane	*Member*
Mrs. Vasumathi Nagarajan	*Member*
Mr. A. Elango	*Member*
Mr. R.S. Mariappan	*Member Secretary*

Committee for prevention of sexual harassment of women in workplace

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. S. Subramanian	*Member*
Dr. V. Vasuki	*Member*
Dr. R. Srinivasan	*Member*
Dr. C. Sadanandane	*Member*
Mrs. Vasumathi Nagarajan	*Member*
Mrs. B. Parassacty	*Member Secretary*

Unit 8
Staff Position

Director

Dr. P. Jambulingam

Scientific

Dr. K. Gunasekaran	*Scientist - G*
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. (Mrs.) V. Vasuki	*Scientist - E*
Dr. R. Srinivasan	*Scientist - E*
Dr. Vijesh Sreedhar Kuttiatt	*Scientist - E*
Mrs. A. Srividya	*Scientist - D*
Dr. C. Sadanandane	*Scientist - C*
Dr. (Mrs.) B. Nandha	*Scientist - B*
Dr. D. Panneer	*Scientist - B*

Administration & Accounts

Mrs. B. Parassacty	*Administrative Officer*
Mrs. Vasumathi Nagarajan	*Accounts Officer*
Mr. S. Balasubramanian	*Section Officer*
Mr. U. Pream Desingh	*Section Officer*
Mrs. T. Ahila	*Assistant*
Mr. Vidjeacoumar S. Raymond	*Assistant*
Mrs. D. Indumathy	*Assistant*
Mr. R. Janarthanan	*Assistant*
Mr. P.N. Ninan	*Assistant*
Mr. N. Suresh Kumar	*Assistant*
Mr. R. Sathish Kumar	*Assistant*
Mrs. J. Kalaiselvi	*Personal Assistant*

Technical

Mr. A. Elango	*Technical Officer - B*
Mr. G. Jeeva	*Technical Officer - A*
Dr. (Mrs.) K. Athisaya Mary	*Technical Officer - A*
Dr. R. Natarajan	*Technical Officer - A*
Mrs. Smrutidhara Dash	*Technical Officer - A*
Dr. (Mrs.) A. Krishnakumari	*Technical Officer - A*
Mrs. Abidha	*Technical Officer - A*
Mr. T. Vijayakumar	*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)*
Mr. K. Harikishan Raju	*Technical Assistant (Research)*
Ms. T. Sankari	*Technical Assistant (Research)*
Mr. S. Muthukumaravel	*Technical Assistant (Research)*
Mr. N. Krishnamoorthy	*Technical Assistant (Research)*
Mr. A. Mathivanan	*Technical Assistant (Research)*
Mr. S. Kandasamy	*Technical Assistant*
Mrs. K.P. Amju	*Technical Assistant*
Mrs. Regnakumari Packirisamy	*Technical Assistant*
Mr. Md. Mustafa Baig	*Technical Assistant*
Mrs. T. Sonia	*Technical Assistant*
Mr. R.S. Mariappan	*Technical Assistant*
Mrs. T. Sumathy	*Technical Assistant*
Mr. Sana Prasad Rao	*Technical Assistant*
Mr. S. Agatheeswaran	*Technical Assistant*
Mr. A.M. Bazeer Ahamed	*Technical Assistant*
Mr. K. Sunil Babu	*Technical Assistant*
Mr. S. Gopalakrishnan	*Technical Assistant*
Mr. M. Sundharesan	*Technical Assistant*
Mr. B. Vijayakumar	*Technical Assistant*
Mrs. G. Vijayalakshmi	*Staff Nurse*

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

*Retired from service on superannuation

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