Vector Control Research Centre

Puducherry

Annual Report 2013

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

VECTOR CONTROL RESEARCH CENTRE (ICMR)
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India
Indira Nagar, Puducherry - 605 006
Tel: 0413-2272396, 2272397 & 2272948 Fax: 0413-2272041
email: vcrc@vsnl.com web:http://www.vcrc.res.in

VECTOR CONTROL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

PUDUCHERRY

Annual Report

2013

CONTENTS

PREFACE

Keeping in view of the National target of lymphatic filariasis elimination, we focused our research mainly on the evaluation of alternative immune-diagnostic tools, and development and validation of xenomonitoring of infection in *Culex* vector and its sampling strategies for surveillance during post-MDA situation. The VCRC joined hands with the Regional Medical Research Centre, Port Blair and the local Health Department in initiating an implementation research on the use of DEC fortified salt as a supplement to mass drug administration to eliminate the lone foci of diurnally sub periodic *Wuchereria bancrofti* in Andaman & Nicobar Islands.

To facilitate vector management and formulation of malaria treatment policies, in the tribal area in Koraput district, Odisha, studies were undertaken to assess the therapeutic efficacy of artesunate combination therapy (ACT) and comparative efficacy of DDT 50% and 75% WDP formulations. Involvement of animal reservoirs in the transmission of cutaneous leishmaniasis was investigated in tribal settlements in Thiruvananthapuram district, Kerala. In view of increasing incidence of Scrub Typhus in the country, efforts have been taken to establish laboratory facilities for diagnosis and to develop expertise on vector surveillance.

Studies on forecasting Japanese encephalitis vector abundance with the aid of Remote Sensing and GIS, and collection of baseline data on JE transmission in Gorakhpur District in order to evaluate feasibility of implementation of vector control measures and its effectiveness for prevention of JE were the other major activities carried out during this year.

During the year, a total of 10 students including a candidate sponsored by the State Health Department, Mizoram were admitted to the M.Sc., PHE Course. Two students from those who completed the course in 2013, were awarded with Internship on inter se merit basis. Both informal and formal training programme continued for the public health personnel and students from various medical / public health Institutions. Biologists and Senior Entomologists (17 Nos.) sponsored by NVBDCP from different States of our country were trained on the epidemiology and control of vectors and vector-borne diseases.

The fund allocation for research and other important activities of the Centre continued to be low; but, still the Centre managed to sustain its activities 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 of ICMR, New Delhi. The Task Force of ICMR (VBDSF & THRF), National Vector-Borne Disease Control Programme (NVBDCP), New Delhi, Department of Science and Technology (Govt. of India), WHO, Geneva, WHO-SEARO and LF Support Centre-Bill & Melinda Gates Foundation supported various research and training programmes.

The support of Scientific Advisory Committee and other Institutional Committees of the Centre could led to the successful completion of research and other supportive activities. The Directorate of National Vector-Borne Disease Control Programme (NVBDCP), New Delhi, Dept. of Health & Family Welfare, Govt. of Puducherry, Tamil Nadu, Kerala and Odisha, and Rubber Research Institute, Kottayam, Kerala have collaborated with the Centre and extended all possible help in the field research activities of the Centre.

I wish to express my profound thanks to all the scientists and staff of our Centre for their unstinted support and cooperation for the entire achievements accrued.

JAI HIND

Dr. P. Jambulingam
Director

EXECUTIVE SUMMARY

The thrust areas of research of the Centre continued to be on the elimination of lymphatic filariasis and development of tools and strategies to support the National and State Vector Borne Disease Control programmes. The Centre also undertook studies on the re-emerging vector-borne diseases such as Scrub Typhus. The major activities carried out at the Centre during the year are summarized, below:

Lymphatic Filariasis

- Towards developing an antibody assay to detect exposure to filarial infection, six peptides were synthesized and tested. Three showed high reactivity to antigen positive sera, with a sensitivity of 100% and specificity of 90% in the preliminary screening. These candidates are identified as potential markers to be tested and validated further.
- Another peptide to a *W. bancrofti* specific epitope-WbL3PP2 showed immune-reactivity to *W. bancrofti* infection in humans and mosquito vectors. Preliminary testing of 34 coded human sera samples showed only 50% sensitivity and 59% specificity.
- RT-PCR based assay developed at the centre for the detection of infective (L3) stage larvae of lymphatic filarial parasite, *W. bancrofti*, in vector mosquito *Cx. quinquefasciatus* was validated through a multi-centric evaluation at four ICMR Institutes. Results indicate potential application of the assay in monitoring the transmission of LF.
- A prototype of an electrochemical detector of *W. bancrofti* infection in vectors, developed at the centre, is being refined to a miniaturized version in order to make it user friendly. The experiment is showing appreciable results towards DNA hybridization and also it can provide a potential platform for the construction of DNA biosensor.
- A vector sampling design was standardized and PCR based methods of assessing vector infection have been developed to monitor vector infection during post-MDA period. The strategy was validated by repeat surveys at two stages with a gap of two years. The results suggest that vector sampling is highly efficient when compared to human sampling for assessing Mf-prevalence, particularly when human infection prevalence is below 1%. Using this method it was shown that vector infection declined in hotspot areas in an area where more than 8 rounds of MDA have been completed.
- Another study carried out in four communities assessing Ag prevalence and vector infection during post MDA period showed absence of recent transmission in two consecutive surveys, indicating that 1% Mf prevalence as cut-off value was safe to discontinue MDA. The adult age class cannot be targeted for evaluation since the post-MDA Ag-prevalence in adult age group has shown no relationship with that in children.
- Transmission Assessment Survey was carried out in collaboration with the NVBDCP in five evaluation units to assess the impact for stopping MDA. The results showed that the protocol require standardization to our operational settings. Migrant children and children from other implementation units should be excluded from sampling. Data from immunization records is recommended to calculate school enrolment of children. Inflated census data could be an issue to be taken care.

Malaria / Leishmaniasis

- ACT treatment of uncomplicated falciparum malaria cases in Odisha (n = 75) gave good clinical response and the parasite density was reduced by 96% by day 3 suggesting adequate response to artemisinin. Late parasitological failure was observed in about 27% of cases (confirmed by microscopy and PCR) suggesting failure of partner drugs. The adverse drug reactions were mild and abdominal pain was predominant.

- A comparative evaluation of indoor residual spraying of DDT WDP 75% and DDT WDP 50% against the malaria vectors, *An. fluviatilis* and *An. culicifascies* in Odisha has shown a significant reduction in vector density after the first round of spray with no difference between the formulations. However, on one time mud plastered surfaces, the mortality was 98.9% with DDT 75% and 49.1% with DDT 50%.
- The cases recorded with symptoms similar to cutaneous leishmaniasis in the Kani tribal settlements (Kani tribes) in Kerala was found to be caused by *L. Donovani. P. argentipes* and *P. colabaensis,* the implicated vectors constituted about 21% of the total number of sandflies collected.

Dengue / Chikungunya / JE

- The field studies for the development of RS-GIS based Model to Forecast JE Vector Abundance and Transmission Risk showed a significant correlation between crop height and vector densities (adult as well as larvae). The σ0 values as derived from the Indian Satellite data increase with stages of paddy growth suggesting that the backscatter coefficient could be used as a proxy for monitoring paddy growth and possible relationship with JE vector density.
- A research study on vector control to minimize the risk of transmission of JE in Gorakhpur District has been initiated with the objectives of generating detailed information on the bionomics of the vector for extended intervention plan and planning and implementing measures for reducing man-vector contact at block level for JE prevention / control. Baseline data on Vector density, human blood index and vector infection have been collected.

Scrub Typhus

- Laboratory facility has been established for Scrub typhus at VCRC and screening samples of scrub typhus cases reporting to the Medical colleges in Puducherry shows that ISS-11 is the most common genotype identified, others being Inha Kp1186344, CMC Scrub E6, UT219 and CBNU-19.

Microbial / chemical agents for vector control

- A 80 kDa protein was additionally expressed and inherited in *Bacillus sphaericus* - resistant mosquitoes (*Cx. quinquefasciatus*) raised in the laboratory.
- Development and testing of nanoparticle-based formulations of larvicides showed that nano-formulation for mosquito larvicides will have limited application in mosquito control.
- An optimised production medium and downstream process for obtaining the metabolite by the mosquitocidal bacterium *B. amyloliquefaciens* (VCRC B483) have been standardised.

Translational Research

- Two macrofilaricidal combinations MCT-6 & MCT-7 have shown promising macrofilaricidal activity against adult *Setaria digitata* under *in vitro* conditions. However, low activity was observed with MCT-7 when given by oral route in animal models, possibly due to its poor aqueous solubility and bioavailability.
- An improved method of production and downstream processing of Thrombinase, a blood clot dissolving enzyme, from a *Bacillus sphaericus* (strain no. NRRL B 18949) has been standardized. The peptide profile generated a total of 29 peptides ranging from 599 kDa to 2716 kDa and the Molecular Weight of thrombinase was determined to be 28.95 kDa.
- In a preliminary field evaluation against *Cx. quinquefasciatus*, the formulation of *Pseudomonas fluorescens* (VCRC B426) was effective at a reduced dosage of 90 ml / m2 resulting > 90% pupal mortality up to 9 days post-treatment.
- Fermentation process for production of mosquitocidal lipopeptides of *B. subtilis* has been upscaled and the downstream processing techniques optimized. One of the aquoeus formulations prepared and evaluated against the pupal stages of *An. stephensi* was found to be active against mosquitoes.

Human Resource Development

1. Academic

M.Sc. Public Health Entomology:

From the first batch of students who have successfully completed their course, award of internship has been given to two students, based on the inter-se merit list obtained from Pondicherry University.
The syllabus for the course was revised with effect from the academic year 2013–14.

Ph.D. Programmes:

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

Post-Doctoral Fellowship:

One Post-Doctoral Fellow is pursuing his research under the ICMR PDF programme in Chemistry.

Individual Students' Project:

Seven students (Indian University: 3; Foreign University: 4) have undertaken projects, as partial fulfilment of their degree.

2. Training

Formal:

Training course jointly organized by VCRC, Puducherry & NVBDCP, New Delhi. Seventeen Biologists / Senior Entomologists (Gujarat, Haryana, Karnataka, Andhra Pradesh, Tamil Nadu & Puducherry) - participated.

Informal:

Medical Officer (1) and Entomological Assistants (2) from Sri Lanka, Research scholars (5) and PG students (6) from Tamil Nadu were offered training in the field of vector entomology, surveillance, etc.

Observational:

Over 400 students from different Institutes visited VCRC for orientation and exposure to various ongoing programmes of the centre.

1 Scientific Activities

1.1 LYMPHATIC FILARIASIS

1.1.1 Identification and characterization of potential immuno-diagnostic molecules from the L3 stage filarial parasite *Wuchereria bancrofti* for measuring the exposure to infection

IM 1011: Jan 2010 – June 2013

Athisaya Mary K, Paily KP, Hoti SL

Assessment of exposure to LF infection is an important parameter to evaluate filariasis elimination programme. Total interruption of transmission following mass drug administration can be verified from the antibody prevalence among children (age < 5 years) born after intervention. This indicator will also be useful for surveillance during post-MDA period, to check the resurgence of infection. This study was, therefore, undertaken with the following objectives:

- To identify and characterize specific molecules of *W. bancrofti* suitable for detection of human exposure to infective stage of the parasite.
- To assess the potential of specific molecules for measurement of exposure under laboratory level evaluation.

Infective stage larvae of the filarial parasite *W. bancrofti* were raised by feeding *Culex quinquefasciatus* mosquitoes on mf positive blood samples collected from human volunteers. BALB/c mice were immunized with L3 stages of *W. bancrofti* to get L3 anti-sera. When the anti-sera were reacted with L3 stage antigens of *W. bancrofti*, a 43 kDa protein molecule was found to be reacting specifically with L3 stage antibodies.

This 43 kDa protein molecule was subjected to sequencing by LC-MS/MS analysis. From the analysis, six peptides with 20 or more of the amino acids were synthesized (AR 2012) and evaluated for their potential in detecting filarial specific antibodies. Initially they were tested with known positive human sera samples. Peptides P1, P2 and P3, showed 100% reactivity. Further, when they were tested against BALB/c mice sera raised against *W. bancrofti* L3, P1 and P3 showed 100% reactivity indicating that the antibodies are specific to L3 stages. Subsequently, these peptides were tested against 10 endemic normal sera samples. The peptide P1 detected all the 10 samples as positive for filarial specific antibodies (IgG) and the highest O.D value was 0.324 in comparison with 0.073 of non endemic normal serum (NEN) sample. Peptide 2 & 3 could detect 9 out of 10 samples as positives. Hence, the specificity of the assay was found to be 100% for P1 and 90% for P2 & P3.

Further testing of the peptides with more number of non endemic normal sera samples to arrive at a cut off value is required for developing this molecule as a marker to measure the exposure rate.

1.1.2 Immunological evaluation of the sensitivity and specificity of the L3 specific peptide, WbL3PP2 for monitoring filarial infection

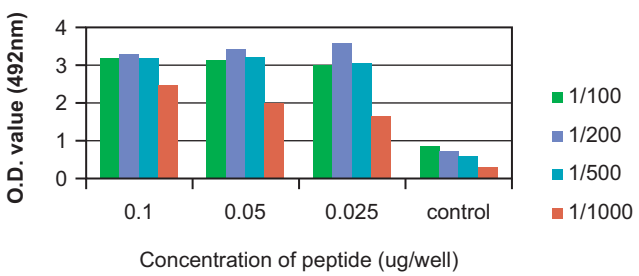
IM 1202: Jan 2012 – Dec 2013

Vasuki V, Subramanian S, Hoti SL, Jambulingam P

In another attempt to develop an indigenous antibody assay, we have identified an epitope (WbL3PP2) which was found to show immunogenicity. The sensitivity and specificity of this diagnostic candidate was assessed against human sera and mosquitoes for monitoring filarial infection.

In order to raise antipeptide (WBL3PP2) antibody, the mice were immunized with each of 200µl (100µl WbL3PP2 + 100 µl CFA) sc/ip for five times at an interval of 10–15 days and post inoculation sera samples were tested for the antibody titre by indirect ELISA. Immunoreactivity of the peptide was tested at three concentrations (0.1, 0.05, 0.025 µg/well) against three dilutions (1:100, 1:200, 1:500) of the anti-peptide sera and all were found to show immunoreactivity (**FIGURE 1.1.1**). The results suggest that a concentration of 0.1µg/well of the peptide is optimum for detecting anti-bodies at 1:200 dilution. Based on these results appropriate concentration of peptide (0.1ug/well) and dilution of human sera (1:200) were used for testing a total of 34 coded human sera samples with minimum three sets of replicates and compared with a gold standard (Og4C3). On decoding, out of 12 antigen positives, six were detected positive for antibody (sensitivity: 50%), while among 22 antigen negatives, 13 were tested antibody negative (specificity: 59%). As per the recommendation of the SAC, the sensitivity of the assay is being validated in two endemic areas involving children age group in the ongoing project.

FIG. 1.1.1 Immunoreactivity of WbL3PP2



1.1.3 Multi-centric evaluation of L3 stage specific RT-PCR assay for the detection of infective stage (L3) *Wuchereria bancrofti* in vector

EM 1201: Jan 2012 – Apr 2013

Hoti SL, Vasuki V, Subramanian S, Sadanandane C

Collaborating Centres: *RMRC, Dibrugargh; RMRC, Port Blair; RMRC, Bhubaneswar; CRME, Madurai*

There is a need for tools that could detect ultra-low level of parasitic infection with high specificity and sensitivity, and high through put for monitoring and assessing transmission during post-MDA surveillance of LF elimination programme. Detection of infective stages of parasites in vectors by dissection and microscopy has been conventionally used for assessment of transmission, but it does not satisfy these requirements for use in the large scale programmes. An infective stage specific RT-PCR assay for *W. bancrofti* was developed at VCRC and validated in the laboratory for stage specificity and sensitivity. Since the assay needs to be independently validated before developing and adopting it further as a monitoring/surveillance tool, a multi-centric evaluation of the RT-PCR assay was carried out at different centres of ICMR with the following objectives.

- To assess the sensitivity and specificity of the infective stage specific RT-PCR assay in detecting infectivity in vectors.
- To evaluate its usefulness under the field conditions at various National Research Centres.

Evaluation of the assay was carried out in three phases and Phase I was monitored by an independent expert.

Phase I: Interactive workshop was conducted where hands on training was imparted to the scientists and technical personnel of the four participating centres on the assessment of sensitivity and specificity of the RT-PCR assay in detecting infective (L_3) stage larvae of *W. bancrofti* in vector. Assay conducted by the participants on limited number of non-coded pooled mosquito samples showed that it is sensitive and specific, while the test on coded pooled mosquitoes containing mixed stages of the parasites showed it to be stage specific detecting the L3 stage even in the presence of other parasite stages.

Phase II: The RT-PCR assay was tested independently by each centre with coded samples of 25 infected pools and 25 uninfected pools of mosquitoes. The results indicated 92–96% specificity and 84–92% sensitivity in two centres and 44–60% sensitivity and 40–60% specificity in other two centres, indicating inter-laboratory variation (**TABLE 1.1.1**).

Phase III: The purpose of Phase III evaluation was mainly to see whether the assay can be employed for detecting infection in wild caught vectors, while also enabling the establishment of the assay in the collaborating centres. A total of 40–100 pools of 25 mosquitoes each (coded by a third party) collected from filariasis endemic areas were subjected to *W. bancrofti* L3 specific RT-PCR assay by each participating centre. Simultaneously, 938–1550 female mosquitoes were subjected to dissection and microscopy for the detection of L_3 stage larvae of *W. bancrofti*. The results from the centres showed that the infectivity rate estimated by PCR assay (0.05%) is comparable to that by dissection method (1.2%) (95% CI overlaps). Centre wise analysis showed that the infectivity rates by PCR assay do not differ significantly from that by dissection method for two of the centres (RMRC, Dibrugarh & RMRC, Port Blair) but for RMRC, Bhubaneswar it differed significantly (95% CI do not overlap) (**TABLE 1.1.2**).

The results from the four participating centres indicated that the assay is as sensitive and stage specific as the conventional mosquito dissection technique. Although the results of Phase II evaluation indicated inter-laboratory variation, the results can be still verified further by the respective centre as they have standardized the technique and established the assay in their laboratories. The assay has been established in centres spread across the country and capacity for performing the assay has been developed. The III phase of the study indicated that the assay has potential application in monitoring the transmission of LF. However, before embarking on such an exercise it may be worthwhile to validate its operational feasibility in areas where there is a need for assessing the transmission.

TABLE 1.1.1 Decoded results of Phase 2 evaluation of 50 samples by RT-PCR assay for detection of infective stage larvae of *W. bancrofti* in mosquito vectors

Name of the Centre	No. of positive pools	No. detected as positive (Sensitivity %)	No. of negative pools	No. detected as negative (Specificity %)	Concordant results*
CRME,Madurai	25	21 (84%)	25	24 (96%)	45 (90%)
RMRC, Dibrugarh	25	23 (92%)	25	23 (92%)	46 (92%)
RMRC, Portblair	25	10 (40%)	25	11 (44%)	21 (42%)
RMRC, Bhubaneshwar	25	15 (60%)	25	15 (60%)	30 (60%)
Overall	100	69 (69%)	100	73 (73%)	142 (71%)

TABLE 1.1.2 Evaluation of the assay on the field collected *Cx. quinquefasciatus* in comparison with dissection & microscopy

Evaluating centre	No. of *Cx. quinque-fasciatus* collected	No. of pools	No. of pools detected as positive	No. of *Cx. quinque-fasciatus* dissected	No. positive for L_3	Infectivity rate (%, 95% CI) by	
						PCR assay	Dissection
RMRC, Dibrugarh	2500	100	19 (19%)	938	13	0.84 (0.5 – 1.3)	1.39 (0.6 – 2.1)
RMRC, Bhubaneswar	1000	40	2 (5%)	1550	29	0.20 (0.02 – 0.7)	1.87 (1.2 – 2.5)
RMRC, Port Balir	1025	42	0 (0)	1000	0	0.0	0.0
CRME, Madurai	Initiated	–	–	Initiated	–	–	–
Overall	4525	182	21	3488	42	0.5 (0.3 – 0.8)	1.2 (0.8 – 1.6)

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

EM 1209: Nov 2012 – Oct 2014

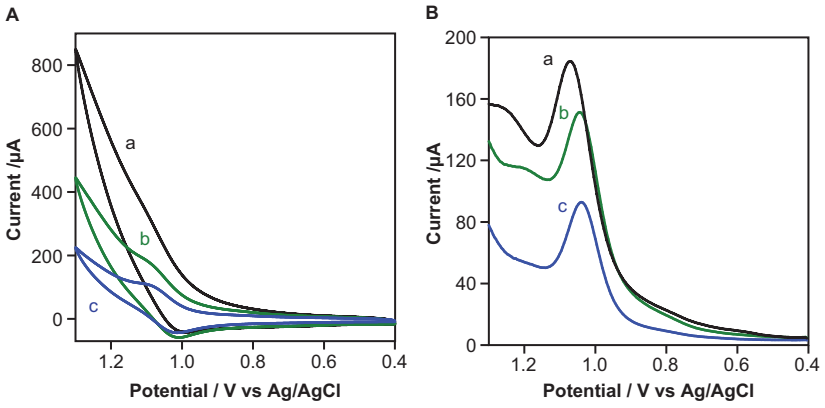
Hoti SL, Vasuki V, Senthil Kumar A

Development of an electrochemical detector has been attempted and a prototype was devised in the earlier project. This prototype needs to be further refined to a miniaturized version to reduce the volume of the analyte and also to make the devise user friendly and portable for the detection of *W. bancrofti* infection in vectors in peripheral areas using screen printed electrodes.

First requisite in the development of biosensor involves the synthesis of specific single stranded (ss) probe DNA of both sense and anti-sense strand of SspI gene of *W. bancrofti*. In order to achieve this, an improved asymmetric PCR was developed, which utilizes single stranded DNA, unlike different proportion of the two strands used in earlier method. The probe DNA so generated was sequenced in an automated DNA sequencer to confirm its identity. The probe DNA was quantified at 260 nm in GenQuant. Further, the 188 base pair probe DNA sequence was cloned into TOPO blunt vector in order to have constant source of probe DNA, without relying on parasite material.

The second requisite for developing electrochemical biosensor by electrochemical method is preparation of suitable chemically modified electrode (CME) to probe DNA hybridization process. Hence, some preliminary work was carried out to choose the right CME and redox mediators for the process. Though various redox mediators such as methylene blue and metal-schiff base complexes etc. have been reported as biomarkers for the hybridization process, strong adsorption on the underlined carbon working electrode surface becomes a major limitation with this type of aromatic system. In order to overcome this limitation, we optimized Ruthenium bipyridyl [$Ru(bpy)_3^{2+}$] as a redox mediator which did not show any adsorption on modified electrode in our study. With this redox mediator, preliminary DNA hybridization work was carried out by using cyclic voltammetry and differential pulse voltammetry techniques with functionalized multiwalled CNT and graphene oxide as CMEs. The **FIGURE 1.1.2** shows the variation in peak currents of $Ru(bpy)_3^{2+}$ at different DNA modified electrodes

FIG. 1.1.2 CV and DPV responses of bare modified electrode (a), Probe ss-DNA (b) and hybridized-DNA with respect to its complementary sequence (c) in presence of $Ru(bPy)_3^{2+}$ in 0.1M PBS with pH 7.4



such as probe ssDNA modified electrode (curve b) 54% and hybridized with complementary ssDNA sequence (curve c) 75% with respect to bare modified electrode (100%). The probed experiment is showing appreciable results towards DNA hybridization and also it can provide a potential platform for the construction of DNA biosensor. Further work is in progress.

1.1.5 National net-work for genotyping of human lymphatic filarial parasite, *Wuchereria bancrofti* from different endemic areas

EM 1137: Nov 2011 – Aug 2013

Hoti SL, Paily KP, Subramanian S, Vasuki V

Collaborating Centres: *RMRC, Dibrugarh (NE); RMRC, Bhubaneswar (East); CRME, Madurai (South); Anna University, Chennai (South); MGIMS, Wardha (Central)*

Filariasis control programme will be dealing with several variants of the parasite, exhibiting polymorphism of genes important for pathogenesis, drug sensitivity and transmission. Therefore, it is essential to fingerprint the genome of the filarial parasite and monitor the dynamics of the genetic structure of population, on a country-wide scale by establishing a network of interested research groups and programme managers. The objectives of the study are to establish a national network of researchers and programme managers interested in the genotyping of *W. bancrofti* and *Brugia malayi* prevailing in endemic areas at different geographic locations and to determine the frequency of alleles of different loci on different genes (*b*-tubulin, Alt 2 and ITS region of rDNA) among *W. bancrofti* parasite populations in different parts of the country.

The collaborating centres carried out surveys for mf carriers in their respective areas and collected blood samples with the approval of the Institutional Ethics Committee. Informed consent from each of the donors was obtained. Details regarding the microfilaremic patients, age, gender and clinical symptoms (if any) were collected from them. The slides were examined for the total number of microfilaria and a maximum of ten microfilariae (per slide) in intact shape and with visible characters were then used for morphometry comprising the measurements of various parameters. Morphometry of 100 mfs have been completed by each collaborating centre.

Isolation and purification of mf from archived blood smears, isolation of genomic DNA from microfilariae, amplification, cloning and sequencing of Alt-2 and ITS region and detection of albendazole resistance by real time PCR have been carried out by the participating centres (**TABLE 1.1.3**). Results from the collaborating centres have been received and subjected to statistical & phylogenetic analysis, and the determination of the frequency of alleles of different loci on different genes (*b*-tubulin, Alt 2 and ITS region of rDNA) among *W. bancrofti* parasite populations from different geographic areas. The results show that the genotyping of the *W. bancrofti* population collected from Wardha region exhibited occurrence of 2–3 sub-population based on 29 bp tandem repeat sequence of marker- Alt2 gene and the genotyping based on beta tubulin gene of *W. bancrofti* indicated the occurrence of albendazole insensitivity alleles among parasite population collected from Wardha (Madya Pradesh) region.

1.1.6 Assessment of long term impact of supplementary strategy with DEC medicated salt in the elimination of lymphatic filariasis

IM 1012: Jan 2009 – Dec 2012

Krishnamoorthy K, Nandha B

Five to six annual rounds of Mass Drug Administration (MDA) with co-administration of DEC and albendazole is the recommended strategy for the control and elimination of lymphatic filariasis. In many countries including India, despite exceeding five rounds of MDA and given levels of coverage, persistent transmission was reported. An attempt was made to agument MDA with mass distribtuion of DEC salt in such an area and assess whether such a strategy could hasten the process of reducing infection levels and interrupting transmission. Through an extramural project a community trial was carried out at block level

TABLE 1.1.3 Work progress in the five collaborating Centres

Centre	Morphometry (No. of mf)	Cloning		Sequencing		β-tubulin RT-PCR			
		Alt2	ITS	Alt2	ITS	Total	A	T	A/T
MIMS, Wardha	100	50	50	50	50	100	93	2	5
RMRC, Bhubaneshwar	100	25	25	20	15	93	46	–	47
CRME, Madurai	100	25	25	*	20	93	86	–	7
RMRC, Dibrugarh	100	8	2	*	*	22	22	–	–
Anna University, Chennai	100	50	50	48	48	25	*	*	*

** Not done*

(MDA + DEC salt arm) with a population of 1.6 lakh in 124 villages and the results were compared between pre and post intervenion. Another block with a population of 1.2 lakh in 88 villages was used as control with continuation of further rounds of MDA alone (MDA arm). One year of supplement of mass DEC salt with MDA could reduce mf prevalence below 1% lending interruption of tranmssion with no new case in children born after the introduction of intervention. In the comparison area, transmission continued despite repeated rounds of MDA. Annual surveys were carried out for four years following the cessation of the intervention to monitor the epidemiological parameters such as community microfilaria prevalence, antigenemia prevalence among children in the age class 2–4 years and vector infection and infectivity. The results showed continued absence of transmission. The study was further extended with intramural funding with the following objectives.

Objectives:

- To carry out background surveillance to verify absence of resurgence of infection, absence of exposure/risk of transmission.
- To assess antigenemia prevalence in children (school entrants) to verify elimination of LF.

Survey in three sentinel sites in each study arm using the sample size determined based on expected microfilaria prevalence (Mf) of 1% has shown that none of the sites in MDA + DEC salt arm (n = 1037) was positive for Mf, while Mf continued to persist with over 1% (range: 1.14 to 2.85%) in MDA alone arm (n = 1203). A total of 880 and 722 children in the age class 5–6 years were sampled for antigenemia in 30 clusters each of MDA + DEC salt arm and in MDA alone arm respectively. ELISA based Og4C3 antigen test was used to detect filarial antigen (Ag). Only in five clusters of MDA + DEC salt area, Ag positive children were found and the overall Ag prevalence was 0.94% (less than the recommended critical level of 1.12%). In MDA alone arm, Ag prevalence was recorded in 62% of the clusters and the overal prevalence was 6.29%, above the critical cutoff, indicating continued transmission despite 9 rounds of MDA alone.

Vector mosquitoes were sampled in seven houses in each of the sentinel sites by using gravid traps for assessing vector infection and infectivity. None of the 167 vector mosquitoes collected from MDA + DEC salt arm was found with any stage of the filarial parasite, showing no evidence for transmission. Dissection of 265 vector mosquitoes showed that 2.6% (range 1.0 to 4.4) and 0.02% of the mosquitoes with filarial infection and infectivity respectively. Vector abundance per trap was 20.4 and 25.3 in MDA + DEC salt and MDA alone arms respectively.

As a final evaluation, the WHO recommended Transmission Assessment Protocol was used to assess the impact of the intervention. Each of the study block (arm) was considered as evaluation unit. School based screening was followed as the school enrolement rate was above 75%. The number of shools to be visited and children to be screened were estimated using Survey Sample Builder version 2.0. Out of 2300 children in 110 schools in the MDA + DEC salt intevention area, 1166 children from 52 schools were screened. ELISA based Og4C3 antigen test was used and only 2 children were found positive for Ag (0.17%) which is below the critical level of 11. Only one cluster was found with Ag positive children. In the comparison area, there were 2251 children in the primary sections of 84 schools. As per the sampling design, 1071 children from 35 schools were screened and found 38 children positive for Ag which was above the critical cut off of 11. The overall Ag prevalence was 3.5%. Out of 35 schools, 15 (43%) were with Ag positive children, Ag prevalence ranging from 1.7 to 21.4% in different schools. The results showed that the intervention (MDA + DEC salt) arm passed TAS, indicating absence of transmission during the past 5 years following cessation of DEC salt distribution.

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

EM 1001: Apr 2010 – Dec 2013

Subramanian S, Sadanandanae C, Vasuki V, Abdul Khader MSM, Krishnamoorthy K, Jambulingam P*

**Institute of Zoonoses and Vector Control, Dept. of Public Health, Hosur, Govt. of Tamil Nadu*

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. This necessitates rapid surveillance tools and appropriate sampling strategies that could identify the low level of infection following multiple annual rounds of MDA. Detection of microfilaria (Mf) in mosquitoes indicates the existence of a reservoir of Mf in the human host while the presence of infective L3-stage larva signifies and quantifies transmission potential. PCR-based assays are found to be more rapid, sensitive and specific for detecting the presence of filarial infection in mosquitoes. The assays could be used for evaluating the impact of filariasis elimination programme and for post-MDA surveillance. However, its application in programme (in place of human sampling) requires sampling methods for mosquito collection.

Objectives:

- To evaluate a mosquito collection sampling strategy that can be used to assess the usefulness of vector infection monitoring by PCR as a surveillance tool for assessing post MDA situation.
- To assess the usefulness of gravid traps for monitoring vector infection in relation to IDR collection by insecticide impregnated fabric traps.

The study was conducted in one of the PHCs (Ammapettai) in Thanjavur district, Tamil Nadu, which had undergone 8 rounds of MDA by 2010 and had two stages: assessing vector infection (i) following 8 rounds of MDA (i.e. in 2010) and (ii) two years after stopping MDA (i.e. in 2012). In each stage two independent surveys were carried out to collect mosquitoes using gravid traps. Briefly the methodology involves collection, identification and preservation of mosquitoes in pools of 25 each for detecting infection by PCR assays. Mosquito sampling was done by fixing gravid traps in selected houses (following systematic sampling procedure) covering all the 33 villages / wards (clusters) of the PHC as well as in 17 streets (clusters) of 4 wards (considered to be 'hotspots' for LF-transmission) in the same PHC. Using two different extraction procedures (crude DNA by VCRC and purified DNA by commercially available qiagen kit) filarial parasite DNA was extracted from parallel mosquito pools of same origin and the DNAs were subjected to quantitative PCR assays.

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

Vector infection: Figures 1.1.3 and 1.1.4 show the results of qPCR assay based on DNA extraction by qiagen and VCRC methods for stages 1 and 2 of the study. In Stage 2, the vector infection rates (95% CI) by qiagen method in surveys 1 and 2 were 0.30% (0.16–0.51) and 0.24% (0.12–0.43) in the PHC clusters (**FIGURE 1.1.3**). The corresponding values for the 'hotspots' in the PHC are 1.55% (1.15–2.03) in survey 1 and 0.50% (0.30–0.77) in survey 2.

PCR assay results based on DNA extraction by VCRC method in stage 2 were 0.68% (0.45–0.97) and 0.46% (0.28–0.72) respectively in surveys 1 and survey 2 in the PHC areas and the corresponding values for 'hotspots' in the PHC are 0.57 (0.36–0.86) and 0.83% (0.56–1.18) respectively (**FIGURE 1.1.4**).

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

FIG. 1.1.3 **Comparison of vector infection rates based on qPCR assay using filarial parasite DNA extractedby qiagen method**

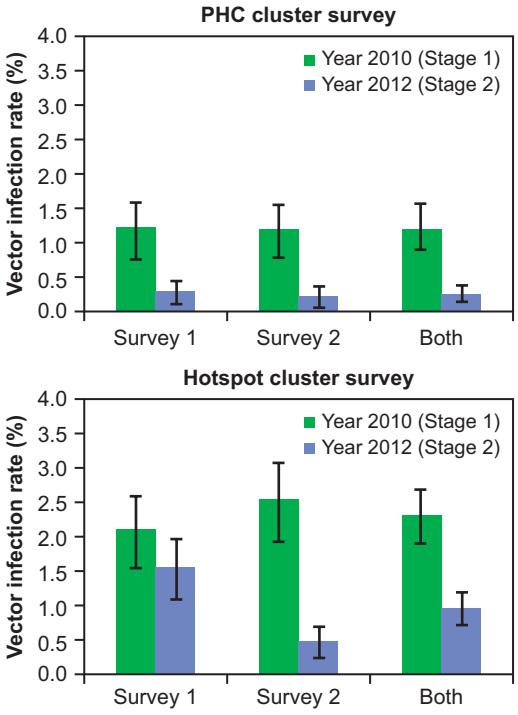
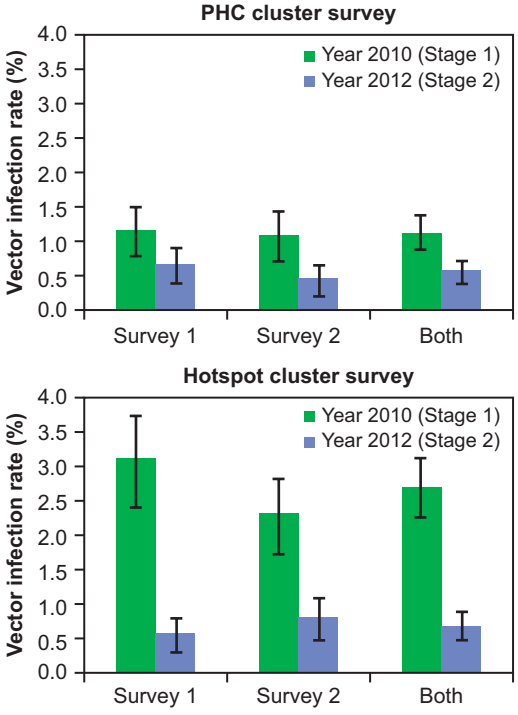


FIG. 1.1.4 **Comparison of vector infection rates based on qPCR assay using filarial parasite DNA extracted by VCRC method**



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

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

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

Vector infectivity: In each stage, a total 207 pools were screen for assessing infectivity of the vector. The pool size varied from 5 to 25 in both stages. A total of 13 (6.3%) and 23 (11.1%) pools were found positive for L3-larave. Pool screen analysis showed that the infectivity rates were 0.26% and 0.51% immediately after 8 rounds of MDA in 2010 and 2 years after stopping MDA in 2012 respectively (**TABLE 1.1.4**). The 95% CIs suggests that the increase in infectivity rate two years after stopping MDA 2012 was not significant (95% CI overlaps).

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

1.1.8 Post-MDA surveillance of rural communities in South India

EM 1016: Nov 2010 – Oct 2013

Krishnamoorthy K

Monitoring the situation after stopping MDA for five more years is recommended before certification of elimination of lymphatic filariasis. During this post-MDA period, the same TAS protocol has to be repeated with a gap of two years. School based survey is recommended when the school enrolment is ≥75%. Otherwise, community based survey is the option which would be operationally difficult. This study was carried out to find whether adult age class can be monitored in place of children and whether monitoring infection in vector population can be an alternate.

Objectives:

- To identify whether stopping the MDA at <1.0% mf rate is safe.
- Whether antigenaemia surveillance of children is appropriate and sufficient or need to be complemented by adult antigenaemia survey or xenomonitoring.
- To monitor the trend in infection levels (gain/loss) in different age groups after stopping MDA and identify the most sensitive indicator for post MDA surveillance in assessing absence of transmission.

The study was carried out in four villages (viz., Kallakulathur, Sithani, Kil-Edayalam and Sendiampakkam) located in Villupuram district of Tamil Nadu, with a population of 5599. These villages received six rounds of MDA, using DEC + Albendazole and further rounds of MDA was stopped as the Mf rate in all the four villages was below 1% with zero level vector infection. The demographic data was updated by house visits. Mass screening of targeted population in two age classes (6–10 & 16–45 years) for filarial antigen using ICT and xenomonitoring with an estimated sample size of 5000 vector mosquitoes were carried out in 2011 and results were already discussed (Annual report 2012). These surveys were repeated after two years in 2013. Mosquitoes were collected using gravid traps from randomly selected houses and were dissected to assess parasite infection. Collections were repeated in all the villages to obtain the required number of mosquitoes.

During 2013, as many as 2989 individuals of the targeted population in 857 households from the study villages were tested for antigenemia. Coverage of children and 16–45 years age classes for antigen test was 75.1% and 71.5% respectively and in all the villages the coverage was above 70%. In one village, none of the children screened in the age class 6–10 years was positive for filarial antigen while in the other three villages it ranged between 1.2 and 1.5%, the overall prevalence being 0.72%. Antigenemia (Ag) prevalence in the adult age class (16–45 years) ranged between 1.2 and 2.2 in different villages, with an average of 1.6%. (**FIGURES 1.1.5** & **1.1.6**)

TABLE 1.1.4 Results of RT-PCR assay for detecting filarial infectivity in *Culex* vector

Stage (Year)	# pools screened	Pool size	# pools positive (%)	95% CI	Infectivity rate (%, 95% CI)
Stage 1 (2010)	207	25–25	13 (6.3)	3.0–9.6	0.26 (0.13–0.45)
Stage 2 (2012)	207	5–25	23 (11.1)	6.8–15.4	0.51 (0.31–0.78)

FIG. 1.1.5 Ag prevalence with 95% C.I. by target age in 2011 and 2013

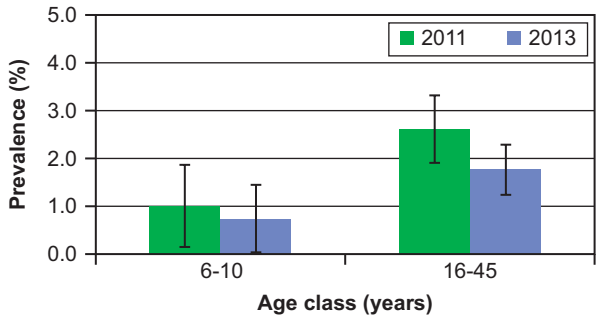
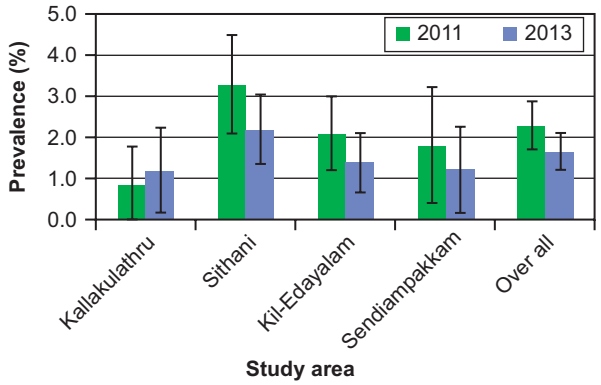
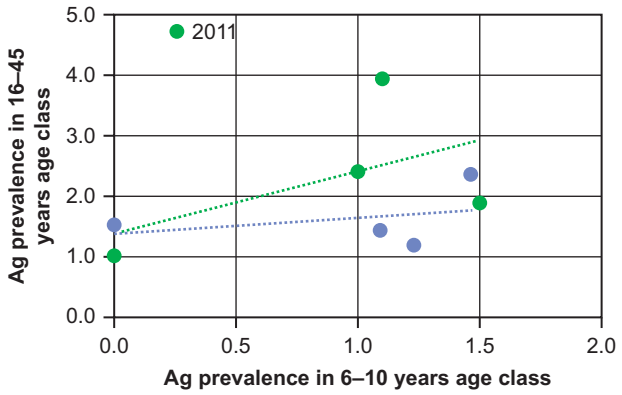


FIG. 1.1.6 Ag prevalence with 95% C.I. by study villages in 2011 and 2013



Comparison of antigenemia prevalence between the two age classes did not show any significant relationship as observed in the previous survey (**FIGURE 1.1.7**). There was about 28% reduction in the Ag prevalence among children and 32.8% reduction among the adult age class, when data were compared with the 2011 survey. However, the reduction was not significant in the children age class ($\chi^2 = 0.23$; $p = 0.63$), while it was at its statistical limit in the adult age class ($\chi^2 = 3.75$; $p = 0.05$). The relative change in Ag prevalence between the age classes was also not significant ($Z = 0.11$; $p > 0.05$), indicating the reduction in Ag prevalence was independent of age.

FIG. 1.1.7 Relationship between Ag prevalence in 6–10 years and 16–45 years age classes



A cohort of 202 children and 934 adults screened during 2011 were followed in 2013. About 1% of the individuals in both the age classes showed gain of infection and about 13.6% of the individuals showed persistence of filarial antigen. The loss of infection was about 86% in both the age classes. There was no positive in both the surveys in the age-class 6–7 years (targeted age-class for TAS) indicating continued absence of transmission even after two years of stopping MDA.

A total of 44 collections using seven gravid traps per village were made covering all the study villages. The traps were set between 1800 hrs and 0600 hours. The number of collections to collect the required sample from each village varied from 7 to 13. The average per trap density was 17 vector (female) mosquitoes. Out of 5282 mosquitoes dissected for filarial infection status, none was found with filarial infection, indicating absence of potential source of filarial infection in the study area during the post MDA period.

Absence of recent transmission in two consecutive post-MDA surveys indicate that 1% Mf prevalence was safe to discontinue MDA. Post-MDA Ag prevalence between children and adult age class is not related, and therefore adult age class cannot be targeted for evaluation. There was no significant reduction in antigenemia prevalence in both children and adult age classes. Xenomonitoring after two years of stopping MDA did not show evidence for vector infection implying absence of potential mf carriers in the study community.

1.2 LYMPHATIC FILARIASIS UNDER TRANSLATIONAL RESEARCH

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

EM 1302: June 2013 – May 2016

RMRC, Port Blair: Shriram AN, Vijayachari

Collaborators: *Directorate of Health Services: B P Saha and Avijit Roy VCRC, Puducherry: Krishnamoorthy K and Jambulingam P*

Programme to eliminate lymphatic filariasis (PELF) using mass annual single dose of DEC with albendazole (ALB) and morbidity management has been launched in India covering all the 243 known endemic districts. Post MDA survey results suggest that more than the recommended 5 rounds of MDA are required to achieve the level of elimination. In the Andaman and Nicobar islands, PELF was launched in 2004 to eliminate filariasis caused by periodic and subperiodic forms of *W. bancrofti*, an important public health problem among the tribal population. Subperiodic form of filariasis is restricted to five islands in Nan Cowry group of islands. The results of Mf survey repeated after six rounds of MDA indicate persistence of infection with 3.3% mf prevalence, with maximum of 5.3% in one of these five islands surveyed. In order to hasten the process of elimination, supplementary inputs are necessary, mass DEC fortified salt has been proposed to be a potential option particularly in island situation where the influx of non-fortified salt can be controlled and could be a supplement to MDA. One year mass distribution of DEC salt (0.2% w/w) has been demonstrated to be effective in reducing mf prevalence below 1% with no new infection among children at a block level trial (population: 1.2 lakhs) in Tamil Nadu (VCRC Annual Report 2010). This was carried out as a supplementary measure to five rounds of MDA. The proposed study is designed as a two arm intervention study to be carried out in collaboration with NVBDCP. Appropriate process and impact indicators have been identified to assess the technical and operational feasibility in the elimination of the lone foci of this infection in India.

Objectives:

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

Specific:

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

A recognizance visit was made jointly by the RMRC and VCRC to the islands selected for the study. There are 43 villages/hamlets in the five Nancowry group of islands with 2756 households and 10460 population. Meetings with local stakeholders including local administration, CHC/PHC, Tribal councils, Captain and local representatives and community was organized. A meeting was held with the Director of Health Services and other investigators of the project and finalised the action plan based on the outcome of the field visit. Questionnaires on salt usage pattern and KAP and data capturing formats for different entomological and epidemiological parameters were pretested. Field surveys have been initiated and covered eight villages in Kamorta island and data on demography, mf prevalence and salt usage pattern have been collected. The study is in progress.

1.3 MALARIA / LEISHMANIASIS / SCRUB TYPHUS

1.3.1 Tolerability, efficacy and operational feasibility of artesunate combination therapy (ACT) (Artesunate-Sulfadoxine + Pyrimethamine): as 1st line antimalaria drug for *falciparum* malaria control in a tribal area in Koraput district, Odisha state

EM 1207: July 2012 – June 2014

Das LK, Krishnamoorthy N, Sahu SS, and Medical Officer i/c, Laxmipur CHC, Koraput, Odisha.

The NVBDCP has introduced artemisinin combination therapy (ACT: Artesunate and Sulfadoxine + pyrimethamine) as 1st line of treatment for uncomplicated falciparum cases in malaria control programme from 2010. This study is being carried out in a stable falciparum area, Laxmipur CHC in Koraput district of Odisha state to monitor the efficacy of this drug combination.

Objectives:

- To evaluate the process of diagnosis and treatment of malaria at community level by ASHA health workers.
- To assess the therapeutic efficacy of ACT (clinico-parasitological response).
- Adverse Drug Reaction (ADR) with ACT, if any.

A total of 93 cases were recruited for the study following inclusion and exclusion criteria. The mean age of the study subjects was 5.3 (±2.3) years. The patients were treated with ACT as per NVBDCP guidelines and supervised consumption was ensured. A total of 75 cases completed 28 day follow-up. Out of 18 dropout cases, 9 refused to participate and the other 9 migrated.

Clinical evaluation: All the study subjects who were administered ACT, were examined for fever, headache, myalgia, nausea, vomiting and other symptoms on days 1, 2, 3, 7, 14 and 28 of post treatment. Serious symptoms and signs were monitored up to day-3 of post treatment. Axillary temperature was measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14 and 28. Temperature was measured with a thermometer that has a precision of 0.1°C. The same route was used throughout the study. The three major clinical symptoms of malaria: fever, headache and vomiting were monitored before and 1, 2, 3, 7 day after medication with ACT. About 3.2% of cases (n = 75) continue to remain mild febrile on 3rd day (**FIGURE 1.3.1**) but recovered on day 4 and hence no medication required.

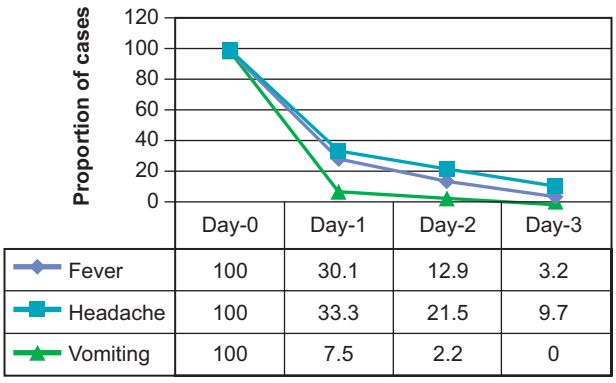
Parasitological evaluation:

Microscopy results: The pre-treatment geometric mean density of parasite was 5563/µl of blood (1200–66200/µl of blood). Parasitological examination by microscopy was carried out on 3, 7, 14 and 28 post treatment days. Treatment outcomes were classified as adequate clinical and parasitological response, early treatment failure, late clinical failure and late parasitological failure as per WHO protocol.

The geometric mean density of parasite was reduced by 96% on day-3 post treatment. However, complete clearance of circulating parasites was observed only in 53.3% of cases by day-3 post treatment (**FIGURE 1.3.2**).

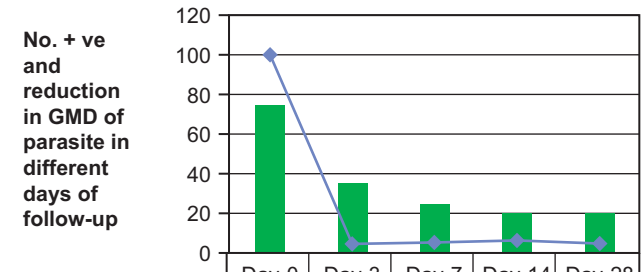
PCR results: Pre and post treatment filter paper blood samples of 36 cases were examined for *Pf* species by PCR technique. Ten cases had parasitaemia (late parasitological

FIG. 1.3.1 **Day wise proportion of cases with fever, headache and vomiting in comparison to pre Rx**



	Day-0	Day-1	Day-2	Day-3
Fever	100	30.1	12.9	3.2
Headache	100	33.3	21.5	9.7
Vomiting	100	7.5	2.2	0

FIG. 1.3.2 **Number +ve and reduction in GMD of parasite in different days of follow up**



	Day-0	Day-3	Day-7	Day-14	Day-28
No.+ve	75	35	25	20	21
GMD of parasite	100	4.116	5.446	6.345	5.284

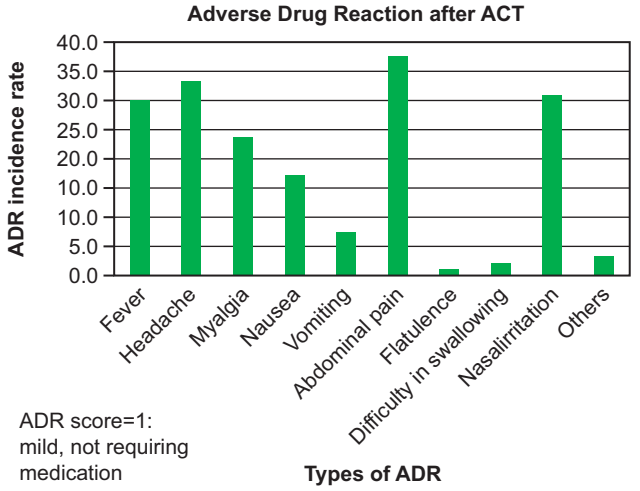
failure) between day 7–28 of which 5 cases were due to recrudescence. There was no difference between microscopy (26.7%) and PCR (27.8%) results.

Adverse drug reaction (ADR): The overall incidence of adverse reaction was 75.27% (70/93). The major symptoms were abdominal pain and nasal irritations followed by fever, headache, myalgia and vomiting. However, fever, headache, myalgia and vomiting could also be a symptom of malaria. Abdominal pain was observed consistently and duration of this symptom remained up to 3 days after starting the treatment and could be related to ACT. The duration of other mild symptoms varied between 1–5 days. The details of other adverse symptom and duration are given in **FIGURE 1.3.3**.

Process of diagnosing malaria by ASHAs: A total of 102 ASHAS were interviewed with a pretested questionnaire on the process of diagnosis and treatment of malaria cases in the villages. Training of ASHAs for malaria control activities at regular interval is required. 60% of ASHAs give ACT, 3–days' doses on 1st day to take in 3 days and only 2% of ASHAs visit fever cases at their door steps.

The drug regimen ACT has good clinical response in uncomplicated falciparum malaria, although the fever resolution is by crisis. Reduction of parasite density by 96% by day 3 suggests adequate response to artemisinin. However, there is late parasitological failure of about 27% of cases studied as observed both by microscopy and PCR suggesting failure of partner drugs: sulfadoxine and pyrimethamine. The adverse drug reactions were mild and abdominal pain was the predominant observation. About 28% ASHAs either do not know or take others' help for performing RDT. 60% of ASHAs give ACT for 3–days' dose on the 1st day of patients' visit and the remaining ASHAs distribute ACT on daily basis.

FIG. 1.3.3 **Types of ADR after ACT (N = 93)**



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

EM 1301: 18 months (2013 – 2014)

Gunasekaran K, Sahu SS, Subramanian S, Kali Prasad Behera, Pradhan MM

DDT, the most inexpensive and common insecticide used for malaria and kala-azar control, is available in two formulations, WDP 50% and WDP 75%. Although, DDT WDP 75% is considered as the cost effective high performance formulation for indoor residual spraying (IRS) with long lasting residual properties, no information is available on the comparative efficacy of these two formulations in Indian conditions. It is essential to have such information to consider replacing DDT WDP 50% with DDT 75% for IRS. Hence, this proposal is to compare the effectiveness of indoor residual spraying with DDT WDP 75% @ one gm/m² and that of DDT WDP 50% @ one gm/m² in controlling *Anopheles fluviatilis*, the primary vector of malaria in the selected endemic areas of Odisha State.

Objectives:

General: To evaluate the comparative effectiveness of indoor residual spraying of DDT WDP 75% @ one gm/m² with that of DDT WDP 50% @ one gm/m² in controlling the malaria vector, *An. fluviatilis.*

Specific:

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

Study area: The study is conducted in Kumbhari and Jogipaluru sub-centres (SCs) of Narayanpatana community health centre (CHC) of Koraput district of Odisha State. DDT WDP 50% is currently in use for indoor residual spraying (IRS) for malaria control.

Study design: This is an intervention study with two arms, one with residual spraying of DDT WDP 50% @ one gm/m² in Kumbhari SC and the other with DDT WDP 75% @ one gm/m² in Jogipaluru SC. Since the trial is carried out in an endemic area where routine malaria control operations are being implemented, it was not possible or ethical to have an additional arm without intervention. The two arms (two SCs) are comparable in terms of ecotypes, human population size and vector density, with vector

density as the main parameter. The two arms were randomized to receive either one of the two DDT formulations. The entomological parameters are monitored in six index villages randomly selected from each arm, in order to evaluate the impact of IRS.

Spray coverage: First round of IRS was carried out in the two SCs (Kumbhari SC: 9 villages; Jogipaluru: 10 villages) between 16th and 25th July 2013. Out of the 1,425 rooms present in the Kumbhari SC, 83.4% were sprayed with DDT 50%; spray coverage varied from 77.1% in Mandiaguda to 98.3% in Rangamguda village. Similarly, of the 1,473 rooms present in the Jogipaluru SC, 86.9% were sprayed with DDT 75%; spray coverage ranged from 77.7% in Pilbary to 99.1% in Khajaguda village.

Second round of IRS was done in the two SCs between 6th and 15th November 2013. In the Kumbhari SC, 75.6% of the total 1,467 rooms were sprayed with DDT 50%; the room coverage varied from 70.3% in Dandabadi to 87.9% in Mandiaguda village. in the Jogipaluru SC, of the total 1,572 rooms 80.1% were sprayed with DDT 75%; the spray coverage in the villages ranged from 70.4% in Pallaput to 99.1% in Gechella.

Mud plastering of rooms: One month after first round of spraying, 22.6% (n = 1425) and 13.2% (n = 1473) of the sprayed rooms were found mud plastered in Kumbhari (DDT 50% arm) and Jogipaluru SC (DDT 75% arm), respectively. After two months of spraying, there was an increase in the proportion of mud plastered rooms in both DDT 50% (27.8%) and DDT 75% (42.9%) arms.

After one month of II round of spraying, the proportion of the sprayed rooms found mud-plastered was 11.6% (n = 1467) and 9.8% (n = 1572) in Kumbhari (DDT 50%) and Jogipaluru SCs (DDT 75%), respectively. At two months post-spraying, the mud plastering coverage was higher in both DDT 50% (27.7%) and DDT 75% (25.1%) arms.

Species composition: A total of 1,778 anophelines comprising 13 species (610 anophelines of 10 species before spraying and 1,168 anophelines of 13 species after spraying) were collected in the two SCs. This included *An. fluviatilis* and *An. culicifacies,* the recognized vectors of malaria and *An. aconitus, An. jeyporiensis, An. maculatus* and *An. varuna*, the known vectors of secondary importance in India. Among the anophelines, the most abundant one was *An. subpictus* (43.3%) followed by *An. culicifacies* (29.5%) and *An. vagus* (13.7%).

Relative abundance of vector(s) resting indoors (Resting density indoors): Prior to spraying, the average per man hour density (PMDI) of *An. culicifacies* indoors in human dwellings of six index villages of Kumbhari SC varied from 0.2 to 5.2 and the corresponding values in Jogipaluru SC were 0.0 to 5.0. After the I round of spraying, the PMDI of *An. culicifacies* varied from 2.3 to 4.0 in Kumbhari and 0.3 to 7.2 in Jogipaluru SC indicating that IRS with both the formulations of DDT did not have any impact on indoor resting density of *An. culicifacies*, which is resistant to this insecticide. After the II round of spraying, the PMDI of *An. culicifacies* was zero up to December 2013 in both Kumbhari and Jogipaluru SCs (**FIGURE 1.3.4**). This was due to the seasonal effect of the winter season.

Before the I round of spraying, the average PMDI of *An. fluviatilis* in human dwellings of six index villages in Kumbhari SC varied from 0.0 to 0.3 and the same in Jogipaluru SC was from 0.0 to 0.7. After first round of spraying, the PMDI of *An. fluviatilis* reduced to '0' and maintained at '0' level up to week 13 post-spraying in both the SCs and on 14th week there was a slight increase in the PMDI in Jogipaluru SC. The results indicated that both the DDT formulations produced a good impact on indoor resting density of *An. fluviatilis* (**FIGURE 1.3.5**). After second round of spraying, the PMDI was at '0' level up to December 2013 (i.e. up to week 17) in both Kumbhari SC and Jogipaluru SC (**FIGURE 1.3.5**).

FIG. 1.3.4 **Indoor resting density (Number per man-hour) of *An. culicifacies* in human dwellings before and after indoor residual spraying with DDT 50% and DDT 75%**

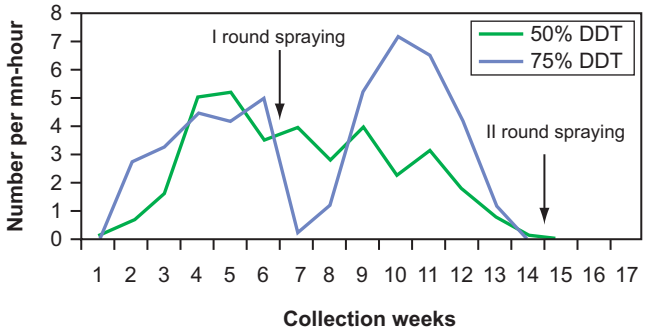
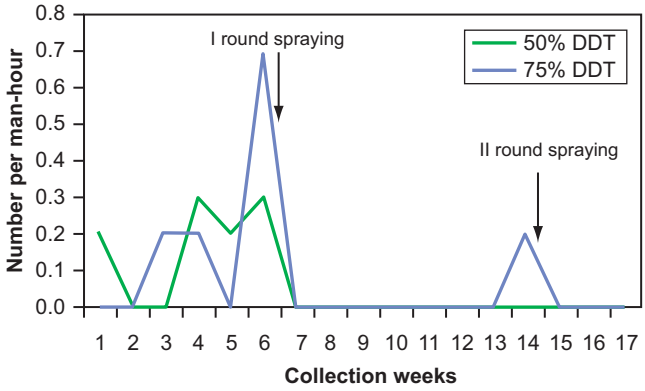


FIG. 1.3.5 **Indoor resting density (Number per man-hour) of *An. fluviatilis* in human dwellings before and after indoor residual spraying with DDT 50% and DDT 75%**



Overall, light trap collections in human dwellings were poor in both the arms. The per trap-night density (PTD) ± SE of *An. culicifacies* in DDT 50% arm was 0.04 ± 0.04 prior to spraying and after first round of spray the PTD was 0.08 ± 0.06. The corresponding values in 75% arm were 0.06 ± 0.02 and 0.18 ± 0.10 indicating no impact of the two formulations of DDT on *An. culicifacies*. After II round of spraying, the PTD in both the SCs was '0' up to December 2013. In the case of *An. fluviatilis*, both the DDT formulations brought down the PTD after first round of spraying; in the 50% arm from 0.20 ± 0.07 to 0.02 ± 0.02 and in the 75% arm from 0.40 ± 0.16 to 0.12 ± 0.07. After II round of spraying, only one *An. fluviatilis* was collected in each SC up to December 2013.

Relative abundance of vector(s) resting outdoors (Resting density outdoors): Outdoor resting collections also yielded a poor sample size. In the 50% arm, the per man hour density outdoors (PMDO) of *An. culicifacies* before and after first round of spraying was not different and in the 75% arm the density was relatively higher after first round of spraying, suggesting no impact of DDT application on this vector species. After second round of spraying, only one *An. culicifacies* was collected in each SC up to December 2013. In the case of *An. fluviatilis*, in 50% arm, impact of spraying was not reflected from the PMDO, but in the 75% arm, after first round of spraying, the density came down from 0.7 ± 0.26 to 0.3 ± 0.15. After II round of spraying, the PMDO of *An. fluviatilis* was '0' up to December 2013 in 50% arm and there was a slight increase in the PMDO to 1.1 ± 0.34 in the case of 75% arm; this increase might be due to the seasonal effect of winter.

Parous rate: Parous rate was calculated from the mosquito samples collected only from human dwellings and used for comparison. There was only a marginal decrease in the parous rate of *An. culicifacies* after first round of spraying in the DDT 50% arm, i.e. from 41.2% (n = 97) to 39.8 (n = 98), whereas in the 75% arm, the decrease was marked, from 54.6% (n = 119) to 29.5% (n = 122). After II round of spraying, parous rate could not be calculated because only one *An. culicifacies* in each arm was collected. The parous rate of *An. fluviatilis* before spraying was 33.3% (n = 6) and 50% (n = 6) in the DDT 50% and 75% arms, respectively. After first round of spraying, up to two months, since there was only zero collection in both the arms, parous rate could not be calculated. After II round of spraying, up to December 2013, only one nulliparous *An. fluviatilis* was collected in 50% arm, and in 75% arm, the parous rate decreased from 29.5% (n = 122) to 5.0% (n = 20).

Human blood index (HBI): Prior to spraying, human blood index (HBI) of *An. fluviatilis* was 0.84 (n = 13) in DDT 50% arm and 0.96 (n = 29) in DDT 75% arm. After first round of spraying, the sample size in 50% arm was too low (n = 1) to comment and in 75% arm the HBI decreased to 0.50 (n = 10). After II round of spraying, the HBI decreased to 0.33 (n = 3) and 0.25 (n = 4) in 50% and 75% arm, respectively. The HBI of *An. culicifacies*, before spraying, was low in both the arms, 0.16 (n = 54) in 50% and 0.08 (n = 75) in 75% arm. After first round of spraying, the HBI decreased to 0.09 (n = 62) and 0.04 (n = 70) in 50% and 75% arm, respectively. After second round of spraying, the HBI could not be calculated as the density was too low in both the arms.

Vector infection rate: In total, 21 *An. culicifacies*, grouped (according to type of collection) in to 5 pools (pool size range: 1–7) and five *An. fluviatilis* in two pools (pool size range: 2–3) were subjected to PCR detection of sporozoites of *P. falciparum* and *P. vivax* in the DDT 50% arm before spraying. Similarly, in the DDT 75% arm, 25 *An. culicifacies* in 6 pools (pool size range: 2–6) and 13 *An. fluviatilis* in 5 pools (pool size range: 2–3) were PCR assayed. Of these, two pools of *An. culicifacies* from DDT 50% arm and one pool of *An. fluviatilis* from the DDT 75% arm were found positive for *P. falciparum*. The maximum likelihood estimation (MLE) of infection rate of *An. culicifacies* and *An. fluviatilis* for *P. falciparum* were 4.7% (95% CI: 0.86 to 15.2) and 5.6% (95% CI: 0.33 to 24.9), respectively. More samples obtained prior to and after spraying are under process.

Residual effect of DDT on the sprayed surfaces: Before spraying, cone-bioassays carried out on wall surfaces in both the arms showed zero mortality of *An. fluviatilis* (n = 20) and *An. culicifacies* (n = 300) indicating absence of insecticide deposit on the walls. Two weeks after first round of spraying, the mortality of *An. culicifacies* was 10.7% in bioassays on DDT 50% sprayed surfaces and 29.3% mortality on DDT 75% sprayed surfaces. Since, *An. culicifacies* was a DDT resistant species and *An. fluviatilis* was not available in adequate numbers, subsequent bioassays were carried out using *An. stephensi.* After one month of first round of spraying, mortality of *An. stephensi* was 98.4% (n = 190) against DDT 50% and 99.5% (n = 190) against DDT 75%. Two months after first round of spraying, while DDT 75% caused a 100% mortality in bioassays, the mortality decreased to 76.7% against DDT 50%. Bioassay was also carried out on sprayed but mud plastered surfaces. After two months of first round spraying, on one time mud plastered surfaces, the mortality was 98.9% with DDT 75% and there was a marked reduction in mortality to 49.1% with DDT 50%.

After one and half months of II round of spraying, the mortality of *An. stephensi* was 100.0% against both DDT 50% (n = 450) and DDT 75% (n = 450) sprayed surfaces and also against sprayed but one time mud plastered surfaces in the two arms (n = 45 and 120, respectively). Filter paper samples with spray deposits and scratched mud samples from the sprayed walls have been sent to the Institute of Pesticide Formulation Technology, Ministry of Chemicals & Fertilizers, Govt. of India, Gurgaon, for DDT content analysis. The results are awaited.

Susceptibility of vectors to common Insecticides: Prior to spraying, the corrected mortality of *An. culicifacies* in susceptibility tests was 9.8% against DDT and 76.1% against

deltamethrin indicating that this vector species was resistant to these two insecticides. After both the round of spraying, the susceptibility status of *An. culicifacies* could not be monitored due to low prevalence. The study is continued.

1.3.3 Entomological and Epidemiological investigations on Leishmaniasis among the Kani forest Tribes in the tribal settlements of Thiruvananthapuram district, Kerala

EM1206: Mar 2012 – Feb 2015

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

Collaborators: *Dilip Kumar (DHS, Govt. of Kerala), Anish TS (Govt. Medical College, Thiruvananthapuram) and Nandakumar S, (DAH, Govt. of Kerala)*

The results of cross sectional survey in the 28 Kani tribal settlements, located in the difficult-to-reach areas of the Western Ghats, Thiruvananthapuram dt., Kerala, on sandfly abundance and cutaneous leishmaniasis (CL) infections were reported in 2012. During the reporting year, a longitudinal survey is being carried out to monitor the seasonal distribution and behavior of sandflies and the extent of CL infection in the tribal settlements.

In the 28 settlements, a total of 768 people, including 341 males and 427 females were examined for CL / suspected infection. A total of 12 human cases, who had healed lesions with scars were noticed in Melaamala (N = 11) and Ayiramkal (N = 1) settlements. Fifteen new CL / suspected cases were recorded from Thazheamala (N = 9), Podium (N = 4) and Kaithode (N = 2) either with nodules or active lesions. Biopsy materials were collected from the nodule / lesion of the 12 new suspected cases, using standard procedure and subjected to histopathological examination. Skin / lesion materials could not be collected from other patients due to other illness. Parts of the specimens collected from each patient were used for PCR assay as well, for parasite identification, at the VCRC Field station, Kottayam, Kerala. In both PCR assay and histopathological examinations, 6 patients were confirmed positive for *Leishmania donovani* and they were given treatment. The number of nodules / papules and or erythematous papules per person varied from 1–8. The lesions were present on the face, upper limb, lower limb, upper part of trunk and other parts. The type of lesions included nodules and nodulo-ulcerative lesions (**FIGURE 1.3.6**). Some of the lesions were bluish red or skin colored. Maximum number of CL / or suspected infections were in the age class i.e., between 10 and 75 years old. Both males and females, including children were affected. Mucosal involvement in lesion form on the upper lip of a patient was recorded as a rare feature in this study (**FIGURE 1.3.7**). Reactivation of one old CL case, with reddish ulcer in the upper pinna after treatment by the local Government Medical College Hospital was observed (**FIGURE 1.3.8**).

FIG. 1.3.6 A nodulo-ulcerative lesion of CL

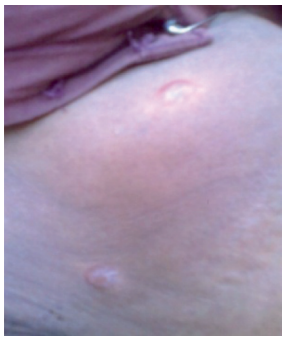


FIG. 1.3.7 A CL case with mucosal involvement

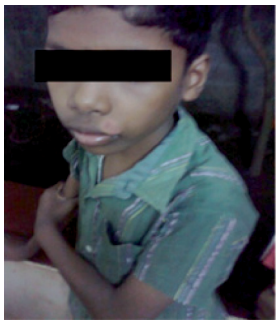
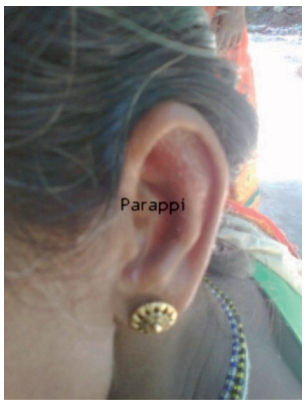


FIG. 1.3.8 Reactivation of a treated CL case



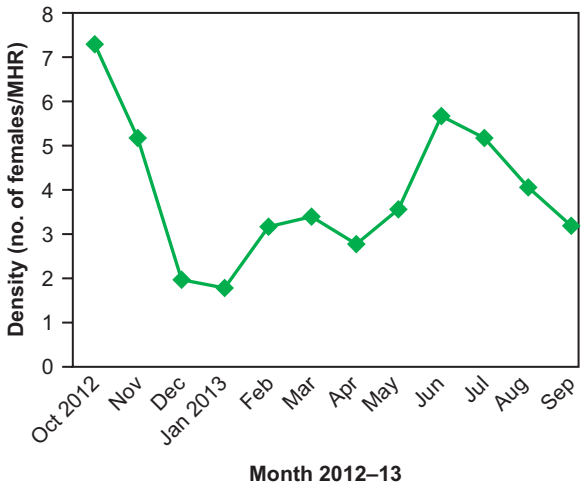
None of the infected tribe had history of travel outside the district, for the past 2–3 years, indicating indigenous transmission of CL infection in this area.

A total of 7837 sandflies, comprising two genera viz., *Phlebotomus* and *Sergentomyia*, constituting 18 species were recorded during the survey (**TABLE 1.3.1**). Of the total, *Phlebotomus argentipes* amounted to 19.16%. The resting density of *P. argentipes* varied from 1.8 (January 2013) to 7.3 (October 2013) females/man hour in human dwellings (**FIGURE 1.3.9**).

TABLE 1.3.1 Sandfly composition (N + 7837)

S.No.	Species	%
1	*P. argentipes*	19.16
2	*P. colabaensis*	2.97
3	*P. stantoni*	1.49
4	*S. baghdadis*	54.65
5	*S. babu*	4.77
6	*S. zeylanica*	4.61
7	*Sergentomyia sp.*	3.67
8	*S. dhandai*	1.80
9	*S. kauli*	1.64
10	*S. rectangulata*	1.56
11	*S. shorttii*	1.33
12	*S. barraudi*	0.86
13	*S. bailyi*	0.47
14	*S. jerighatiansis*	0.39
15	*S. malabaricus*	0.39
16	*S. hospitii*	0.23

FIG. 1.3.9 *P. argentipes* density in human dwellings



So far, 80 females, comprising two species viz., *P. argentipes* and *P. colabaensis* were examined for natural infection and none was positive. Remaining samples (N = 4102) are being examined for *Leishmania* infection. Among the sandflies collected, a total of 189 females were found to be freshly engorged and their blood samples were tested. Of which, 72 were tested to determine the blood meal source, against antisera of rat, dog, human, bird and bovine, using agar-gel diffusion method. It was observed that 75% of the samples showed precipitin formation, towards one or several host blood meals, while the remaining samples were negative. Among females tested, 18.5% were found engorged on human blood. About 40.9% of the females were found to have either double or triple animal blood meals. The results indicate that the blood feeding pattern is complex and there is a possibility of involvement of animal reservoirs in CL transmission in the area.

Historical weather reports of Thiruvananthapuram showed that this area receives rainfall throughout the year with a peak in October. Therefore, most of the months are wet. Although temperature and relative humidity did not show wide fluctuation, temperature was relatively high during March-May and again in September. Sandfly density started increasing just after the onset of monsoon from June and reached a peak in October and declined thereafter. An optimum temperature (~ 28–30°C), high humidity (>80%) and heavy rainfall were found to be congenial for sandfly survival and multiplication.

Further studies to be carried out are: Incrimination of sand fly vector species in CL transmission, determination of role of animal reservoirs in CL transmission and evolving appropriate intervention measures for control and prevention.

1.3.4 *Scrub Typhus:* Establishment of disease and vector surveillance facilities to assess the extent of disease occurrence and vector prevalence

IM 1204: 2011 – 2014

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

Scrub typhus, caused by the organism *Orientia tsutsugamushi* is the most prevalent Rickettsial infection in India and is being increasingly reported from many parts. It is an acute febrile illness which is transmitted by the larvae of *Leptotrombidium deliense*. Humans are infected accidentally and case fatality can be significantly high, if the disease is not diagnosed on time and appropriately treated. In the recent past, there has been a series of outbreaks due to Scrub Typhus reported from various parts of the country indicating the re-emergence of this disease. This project was initiated mainly to establish laboratory facilities for diagnosis and to develop expertise on vector surveillance.

Objectives:

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

During 2012, 70 cases of Scrub typhus were characterized in terms of clinical profile and age distribution. Blood samples have been collected from patients clinically suspected to have Scrub typhus from Pondicherry Institute of Medical Sciences, Indira Gandhi Medical College & Hospital, Kathirkamam, Aarupadai Veedu Medical College and Sri Manakula Vinayagar Medical College, Puducherry.

During the current year, samples from an additional 62 patients have been collected. The clinical profile of a total of 132 patients have been collected and analysed. The clinical profile of these suspected Scrub typhus cases are given in **FIGURE 1.3.10**. Although a majority had only an acute febrile illness with no other complications, some cases also manifested with meningitis and muti-organ disorder. The age group analysis showed that 96 adults and 36 paediatric age-group had scrub typhus.

These cases were from Puducherry and nearby villages and from surrounding Tamil Nadu areas.

The reported cases with fever gradually increased from the mildly hot months of August and September and drastically increased in the cooler months with peaks in December and January to subside in the following months (**FIGURE 1.3.11**).

FIG. 1.3.10 **Clinical profile of Scrub typhus cases (n + 132)**

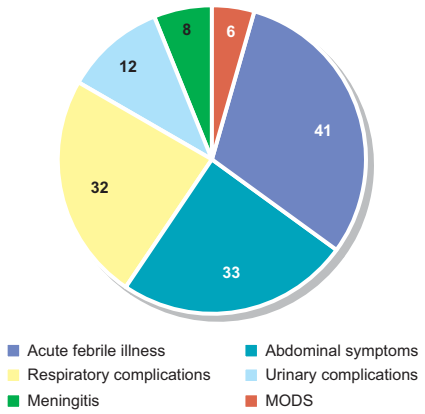
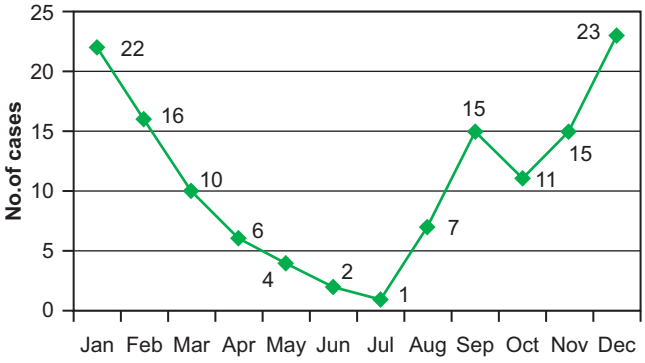


FIG. 1.3.11 **Seasonal distribution of Scrub typhus cases (n + 132)**



Out of 145 samples tested from clinically suspected cases, 115 were anti-56 kDa (Scrub typhus) IgM positive. Further, diagnostic assays were done on these samples and the tests included the Weil-Felix test and Polymerase Chain Reaction to detect 56 kDa antigen, 16s rDNA and GroEL genes of *Orientia* (**TABLE 1.3.2**) & (**FIGURE 1.3.12**). Among the two immunological tests the IgM ELISA tested highest number of samples positive, almost twice as much as the conventional Weil-Felix test. Of the three PCR assays tested, the one based on detection of GroEL gene yielded significantly higher number of positives.

All three the molecular markers have been confirmed as that of *Orie ıtia tsutsugamushi* by nucleic acid sequencing.

Sequencing of samples to determine Genotypes: Determination of genotypes was done on positive samples in 56 kDA PCR, by nucleic acid sequencing. The sequences so obtained were BLASTed for similarity in the NCBI website. Twenty one samples out of the 34 positive samples have been sequenced so far and the genotypes that have been identified are presented in **TABLE 1.3.3**. A total of 6 genotypes were identified and most of the samples (11) belonged to genotype ISS–11.

Phylogenetic analysis of the genotypes have shown that some sequences are more similar to the Madhya Pradesh genotype and a few sequences are of genotypes unique to Puducherry (**FIGURE 1.3.13**).

Vector surveillance: Surveillance of *Leptotrombidium* mites (Chiggers), the vector of scrub typhus was carried out in areas where confirmed human cases of scrub

TABLE 1.3.2 **Comparison of diagnostic tests for Scrub Typhus**

Total No. of samples tested	Positive by				
	Weil-Felix *	IgM ELISA	56 kDa PCR	16s rDNA PCR	GroEL PCR
145	62	115	34	26	63

**Significant titre for Weil-Felix test was taken as 1:160 and above*

FIG. 1.3.12 **Amplification of GroEL gene of Orientia**

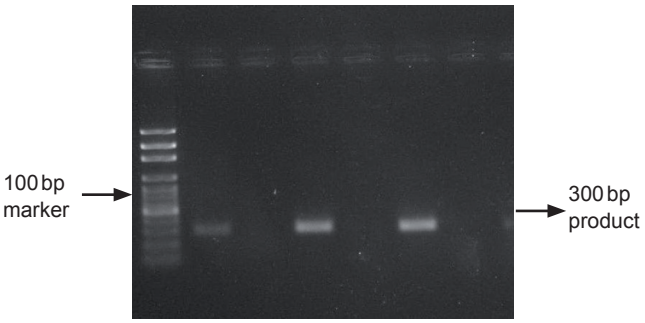


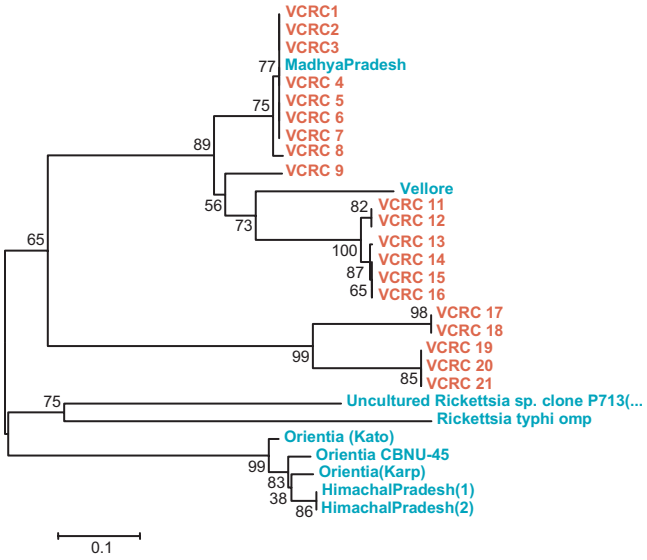
TABLE 1.3.3 Results of Genotyping of *Orientia tsutsugamushi*

S.No.	Genotypes identified (Accession Number)	No. of sequences
1	Uncultured Rickettsia sp. (Vellore)	1
2	CMCScrubE6	3
3	UT219	3
4	ISS-11	11
5	CBNU-19	1
6	Inha-Kp1186344	2

typhus reported. Mites were collected directly from the rodents/shrews. Rodents/shrews were trapped by Sherman live traps (7.6 X 8.9 X 22.9 cm) baited with peanut butter placed between 2 saltine crackers. Trap collections were made in twelve localities belonging to four villages of Puducherry from 18th September to 8th November 2013. All the study locations were characterized by the presence of shrub and bush vegetation. A total of 391 mites were collected from 35 rodents/shrews trapped. *Leptotrombidium (L) deliense*, the vector of scrub typhus constituted 42% of the total mites identified (n = 178).

Scrub typhus continues to cause an acute febrile illness in this region as evidenced both by the antibody detection and the detection of the DNA of the bacterium. ISS-11 is the most common genotype identified so far along with other genotypes like Inha Kp1186344, CMC Scrub E6, UT219 and CBNU-19.

FIG. 1.3.13 Phylogenetic tree constructed from obtained 56 kDa sequences of Orientia (VCRC) strains



1.4 DENGUE / CHIKUNGUNYA / JAPANESE ENCEPHALITIS

1.4.1 Ecology and population dynamics of dengue/chikungunya vectors towards development and demonstration of Integrated Vector Management strategy in Kerala

IM 0716: July 2008 – June 2013

Jambulingam P, Kalyanasundaram M, Sabesan S, Krishnamoorthy K, Pradeep Kumar N, Rajendran G, Vanamail P

The project was initiated at the request of Rubber Research Institute, Govt. of India and in collaboration with the Department of Health Services, Govt. of Kerala in the wake of the large scale outbreak of Chikungunya in Kerala state during 2007.

The objectives of this completed project were:

- To study the vector population dynamics in rubber plantation areas.
- To delineate environmental factors associated with buildup of high density of vectors.
- To monitor infection with dengue and Chikungunya
- To assess socio-cultural and behavioral factors and plantation practices that facilitate vector breeding.
- To develop and demonstrate an integrated IVM strategy for the prevention of *Aedes* transmitted diseases.

Topographically, Kerala state comprises of about 14.0% coastal lowlands and about 28.8% thickly forested highland regions (Western Ghats) with an elevation ranging from 800m-2700m. In between, lie the semi-forested mid highland region with an elevation of 300–700m. Cash crops such as rubber, coffee, coco, pineapple are cultivated in this region as either large scale or small scale plantations. About 5.42 lakh ha of rubber plantations (with 450 trees on an average per ha) and about 30,000 ha of pineapple plantations are cultivated in the mid-highland region. The project was carried out in Kottayam District located in mid-highland region.

Entomological collections were also carried out in Allepey District in the coastal region, one of the chickungunya affected areas for comparison. A total of 32 species of mosquitoes was recorded in the study areas in mid-highland region. *Aedes albopictus* was found to be the most predominant mosquito species (61.70%), which was subsequently incriminated as the vector species of chikungunya virus. However, in the coastal region, Mansonioid species was found to be predominant while *Ae. albopictus* constituted only about 5.0% of the mosquito population sampled.

Latex collection containers [both discarded ones (65.94%) and unused ones fitted to these trees (11.98)] were found to be the major and perennial breeding habitats in rubber plantations; Leaf axils were the major breeding habitats in pineapple plantations and fallen leaves accumulated with rainwater were the breeding habitats in areca nut plantations (**FIGURE 1.4.1**).

The peak of abundance of the vector species, *Ae. albopictus* was recorded during pre-monsoon months in the mid highland region. The pupal index reached to a peak of 49.17 during the month of May.

Abundant intermittent rainfall (about 28.0%) and prevailing plantation practices (such as removal of rain-guards from trees) during pre-monsoon season favored prolific breeding of the vector species. Total rainfall ($t = 2.86477$; $P = 0.0064$) and number of rainy days ($t = 3.41516$; $P = 0.00140$) had a significant positive correlation, with a lag of two fortnights, while temperature recorded a directly significant ($P = 0.0380$) positive correlation with the vector density.

Seroprevalence of CHIKV infection (**FIGURE 1.4.2**) in the community was estimated to be about 68.0% in the rubber plantation areas.

Based on the investigations carried out (described above) community oriented IVM strategy was developed towards control of *Aedes* vectors in two worst affected regions (one village in Kottayam District and another in Pathanamthitta District representing large and small scale plantations of rubber plantations in Kerala).

FIG. 1.4.1 Larval habitats of *Ae. albopictus*

Broadly, the strategies followed in the large scale organized plantations included breeding source reduction/elimination activities by the plantation workers, NSS volunteers of educational institutions and community volunteers as Kudumbasree/self help groups etc. In small scale plantation sector, the entire households in the village were involved in the source reduction activities as groups and the Rubber producer's society (a permanent set up in all the plantation villages across Kerala) was identified as the nodal agency to co-ordinate their activities.

Local communities and other voluntary agencies were motivated by PRA techniques to involve in mosquito habitat reduction activities. Once a week, the household members removed unused latex collection containers in their plantation fields (**FIGURE 1.4.3**). In addition student community, voluntary agencies, religious organizations, educational institutions etc. were also involved in source reduction activities.

In both the villages breeding in tree holes were managed either by filling them or by introduction of *Romanomermis iyengari,* which was found to be effective in controlling *Aedes* breeding. Also, personal protection measures were encouraged among plantation workers, initially by distributing formulations of DEPA which could be directly applied to the exposed skin, which gave a protection from mosquito bites up to 6 hours.

A three tier impact analysis (by the community which gave a feedback to the stakeholders itself, by independent teams and by VCRC) of the program was carried out after one year period of implementation. The results showed a significant reduction of vector population in both the villages (**FIGURES 1.4.4** & **1.4.5**). The *Aedes* pupal indices in both the villages reduced drastically (97.4% in small scale plantations and 81.68% in large scale populations). Checking the spurt of vector population during pre-monsoon months, the identified main risk factor for the outbreaks of arbo-viral diseases was achieved in the post-IVM implementation years in both the categories of rubber plantations. No incidence of any arbo-viral disease (Chikungunya or Dengue) was recorded in the IVM demonstration areas during the project period.

RT-PCR diagnostic facility was extended for arbo-viral disease infections (DENV, CHIKV & JEV) at the request of District and State Health Departments. A total of 92 CHIKV samples and 49 DENV samples were detected from different Districts of Kerala and genetically characterized.

FIG. 1.4.2 **Indirect Immunofluorescence Test for detection of IgG test infection (A – CHIKV Positive: B – CHIKV negative)**

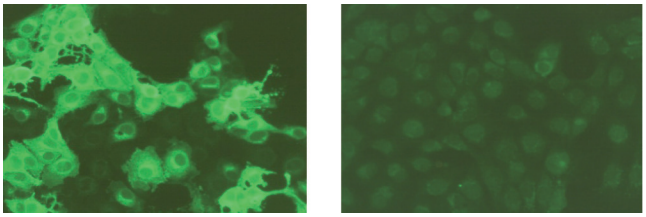


FIG. 1.4.3 **Clearing vector breeding sources by the community volunteers**

FIG. 1.4.4 **Pugal indices through different years**

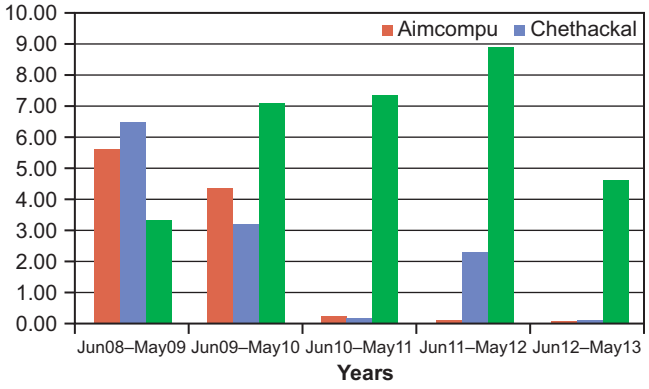
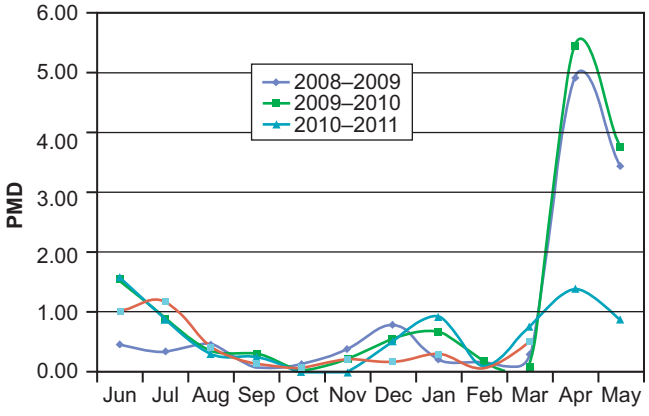


FIG. 1.4.5 **Adult density of *Ae. albopictus* through different years**



1.4.2 Development of RS-GIS based Model to Forecast JE Vector Abundance and Transmission Risk

EM 1130: March 2011 – June 2014

Sabesan S, Rajavel AR, Raju KHK, Subramanian S, Jambulingam P

Collaborating Institutes: *CRME, Madurai (Thenmozhi V), NRSC, Hyderabad; State Public Health Departments: Tamil Nadu & Karnataka*

Objectives:

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

The results of preliminary analysis of vector abundance in relation to paddy growth during 'kharif' season, was presented in our annual report 2012. The field survey continued in 2013 to monitor vector abundance and paddy growth in JE endemic areas namely Cuddalore villages in Tamil Nadu and Bellary villages in Karnataka simultaneously at fortnightly intervals.

Besides achieving the 1st objective of the study, the focus during the current year was on linking RS imageries with different stages of paddy growth.

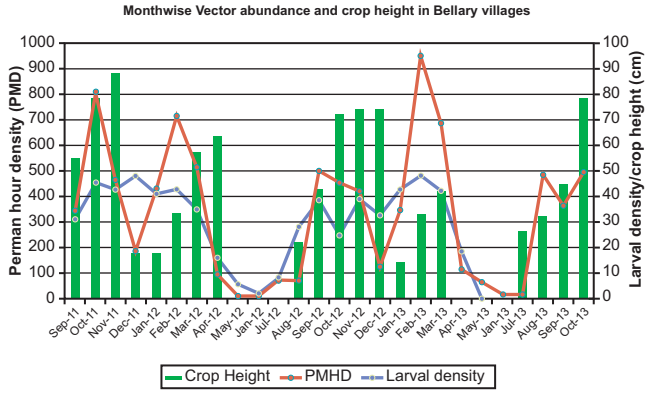
Vector abundance and paddy growth stages: In both Cuddalore villages (Tamil Nadu) and Bellary villages (Karnataka), the increase in adult vector density (*Cu. tritaeniorhynchus*) was observed along with paddy growth up to flowering stage, and then it dips when the crop reached the maturing stage both during 'kharif' and 'rabi' seasons. The occasional vector, *Cx. gelidus* was also recorded in high numbers through both seasons in Cuddalore villages. The immature density of JE vector showed a similar trend as that of adult population in both the study sites (**FIGURE 1.4.6**).

Statistical analysis revealed a significant correlation between crop height (on log scale) and adult ($r = 0.83$, $P < 0.001$) as well as larval density ($r = 0.71$, $P = 0.005$).

Vector infection: Vector mosquitoes in pools (each pool with an average of 50 Nos) were screened for JE virus, using antigen capture ELISA tests. During the study period, the vector infection was recorded in most part of the year. The mean values of minimum infection rate (MIR: No. positive per 1000 mosquitoes) for Bellary and Cuddalore were 1.3 (47 pools +ve / 763 pools tested) and 1.5 (31/521) respectively. Since the MIR is likely to be an underestimation when the infection rate is high and / or the pool sizes are variable, the infection rate was estimated following a maximum likelihood estimation (MLE) procedure. The results showed that the trend in vector infection rates (**FIGURE 1.4.7**) is qualitatively similar to that of MIR but differed quantitatively. In addition, the upper 95% confidence interval for the rates suggests that the rates are highly variable in some months yet the viral activity continues to occur throughout the study period in both areas.

RS-imageries and paddy growth stages: The Indian Satellite [RISAT-1 dual polarization (HH, HV) MRS and FRS data] data available for the last one year are used for identifying the different stages of paddy growth in the study villages. Rice, being a semi aquatic crop, generates unique backscatter profile. Discrimination of different temporal stages of paddy growth is achieved by calculating back scatter coefficient (σ_0) derived from the RS imageries. The σ_0 values were compared with crop height in respective months to identify the spectral signatures specific to different paddy growth stages during the 'kharif' season (**FIGURE 1.4.8**).

FIG. 1.4.6 **Month-wise vector abundance (adult & immature density) and crop height: (a) Bellary, (b) Cuddalore**



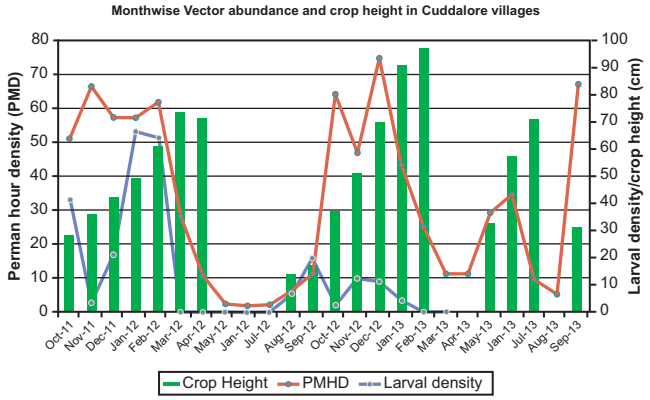


FIG. 1.4.7 Month-wise vector infection rate (MLE method) in the study areas: (a) Bellary, (b) Cuddalore

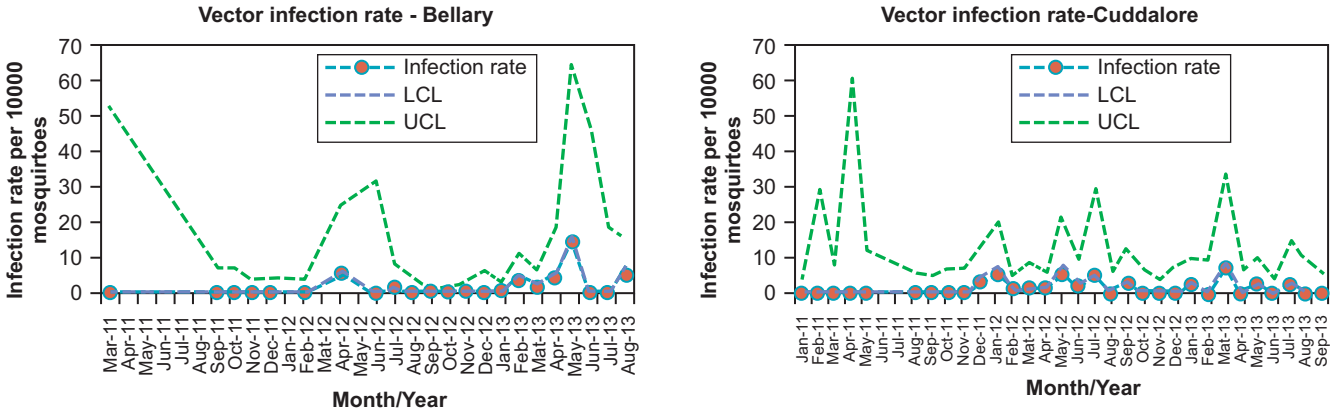
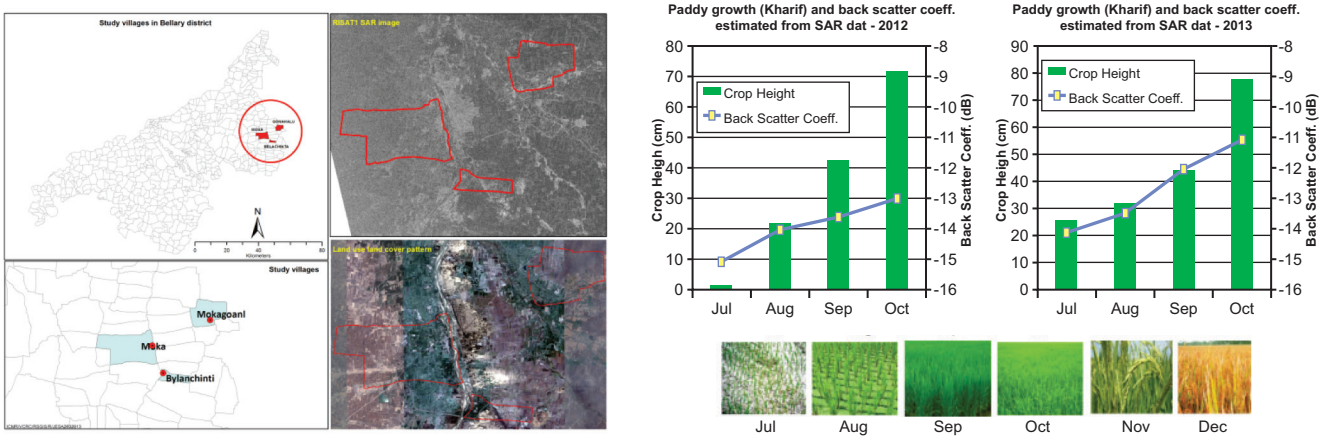


FIG. 1.4.8 Satellite imageries compared to paddy growth stages



The σ_0 values increase with stages of paddy growth suggesting that the backscatter coefficient could be used as a proxy for monitoring paddy growth and hence can be used to explore its possible relationship with JE vector density. For 'rabi' season too, a similar exercise is being done to verify the consistency of satellite data corroborated for different stages of paddy growth.

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

EM 1208: 3 years

Jambulingam P, Rajavel AR, Subramanian S, Gunasekaran K

Objectives:

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

Regular surveys for collection of entomological data were commenced in July 2013 in all the study villages. Fortnightly collections were made in the villages Bahadurpur, Bharsi, Barigaon, Harpur and Gayghat of Belghat intervention block, villages Sarpatha, Machligaon, Ramnagar, Shivpur and Kaharpurwa of Campierganj intervention block of District Gorakhpur and in Katrari, Pidara, Lahilparkhas, Babhani and Saraura in the comparison Block Majhgawa of District Deoria. Indoor resting collections were done in fixed catching stations of human dwellings and cattle sheds and in outdoor habitat in each of the study village for determining the per man hour density. Unfed females were pooled for determining the Minimum Infection Rate (MIR) and stored in -80°C. Blood meal samples were taken on filter paper from fully engorged females for determining Human Blood Index (HBI). Both the samples were transported to the laboratory at VCRC for processing and analysis.

The per man hour resting density of the vector *Cu. tritaeniorhynchus* in the intervention blocks Campierganj & Belghat and in the comparison block Majhgawa is given in **FIGURE 1.4.9**. The per man hour resting density of the vector *Cu. tritaeniorhynchus* in different resting habitats of the intervention blocks Campierganj & Belghat and in the comparison block Majhgawa is given in **FIGURES 1.4.10** & **1.4.11**.

The overall per man hour resting density of the vector *Cu. tritaeniorhynchus* (**FIGURE 1.4.12**) during the period of July – December exhibited a similar trend in all the three blocks with the highest per man hour density recorded in the months of September and October following which there was a decline in the months of November and December. In all the three blocks the indoor resting density was higher in the cattle shed compared to that in the human dwelling (Figs. 1.4.11–13).

A total of 770 blood meal samples from engorged females were collected from the study villages for determining HBI. Out of 286 samples that were subjected to agarose diffusion method, 93% were positive for bovine blood, 1.75% for pig, 0.7% for mixed (human and bovine). Human blood index (HBI) 0. Among the 75 pools (1535 specimens) processed for determining the vector infection rate, 7 pools were were found to be RTPCR-JE positive showing a MIR of 0.46.

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

IM 1303: Sept 2013 – Aug 2016

Pradeep Kumar N, Pradeep Kumar AS, Vijaya Kumar KN, Jambulingam P

Incidence of Dengue fever is on an increasing trend in Kerala (**FIGURE 1.4.13**) and the last year (2013) reported the maximum number of cases (7587 till Oct). Kerala state with an area of about 1.5% of the country and about 2.8% of the population remains worst affected by Dengue and contributes to more than 13.0% of the Dengue cases reported in India.

FIG. 1.4.9 **Per Man Hour Density (PMD) of *Culex tritaeniorhynchus* in intervention and comparison blocks**

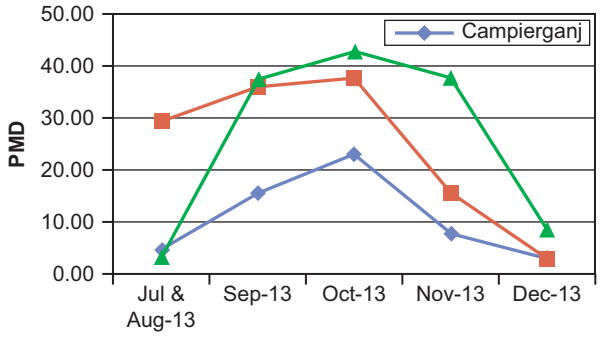


FIG. 1.4.11 **Per Man Hour Density (PMD) of *Culex tritaeniorhynchus* in different resting habitats in Belghat**

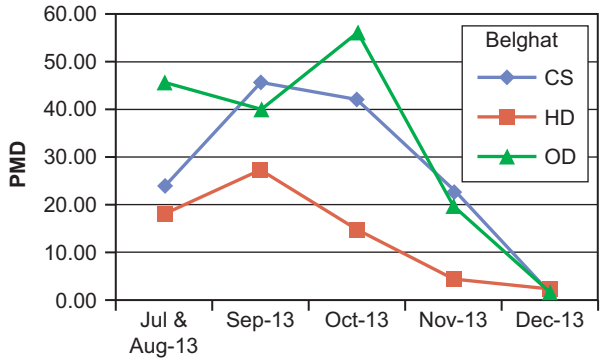


FIG. 1.4.10 **Per Man Hour Density (PMD) of *Culex tritaeniorhynchus* in different resting habitats in Campierganj**

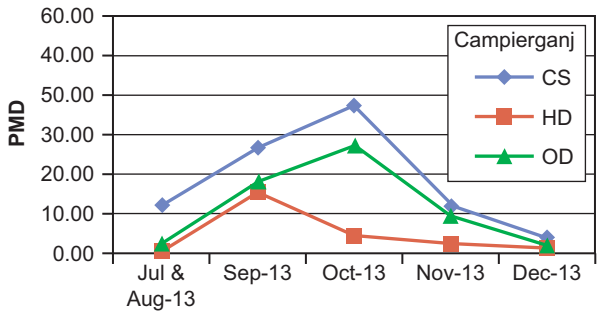


FIG. 1.4.12 **Per Man Hour Density (PMD) of *Culex tritaeniorhynchus* in different resting habitats in Majhgawa**

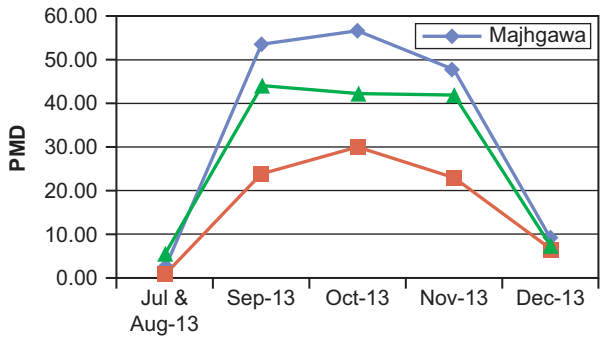
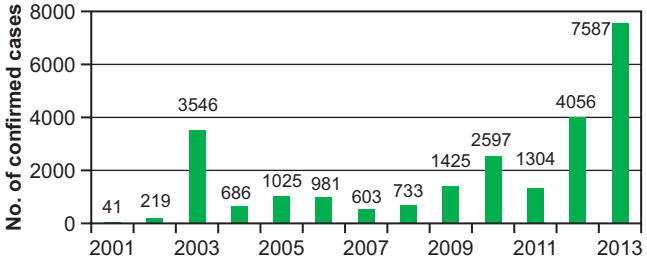


FIG. 1.4.13 Dengue cases reported in Kerala



Trivandrum District contributed to about 56% of the cases. The 2nd worst affected District is Kottayam with a population of 19.79 Lakhs. This District consists of 5 administrative taluks and is bordered by Western Ghats on the eastern side. Kanjirappally and Meenachil taluks on the eastern border is semi/thickly forested and consist of entirely midlands and highlands. The first confirmed case of Dengue in Kerala was reported from Kanjirappally taluk during 1997 and this region continues to contribute Dengue cases every year since then.

The present study is aimed at studying the factors which cause this epidemic spurt of Dengue fever during pre-monsoon season. The study area proposed is located on the border zone of Western Ghats (Kanjirappally Taluk - pop: 269,859), which contributed maximum number of Dengue cases in the Kottayam District of Kerala. Three villages of the taluk, namely, Kanjirappally (pop = 42, 952), Koruthode (pop = 18, 187) and Erumeli (pop = 52, 997) located in the forest fringes of Western Ghats and which were worst affected by Dengue were selected for the study. These 3 villages contributed about 80.0% of DENV cases in the taluk.

Objectives:

- To study relative abundance and population density of Aedine vectors and to delineate their vector breeding habitats in the forest fringe areas.
- To analyze the blood-meal source of vector mosquitoes and to monitor viral activity in vector population.
- To assess sero-prevalence of DENV in human population for estimating the magnitude of dengue transmission in the area.
- To demonstrate IVM to prevent/contain of dengue outbreaks in this ecosystem.

Brief Methodology:

- Fortnightly Entomological surveys are carried out using adult resting collections (both indoor and outdoor) following standard methodologies (Focks, 2003) to monitor vector distribution.
- Indoor resting collections are made by 6 persons for an hour (in ~24 houses spending approximately 15 minutes in a house by an insect collector) to estimate mosquito population densities resting indoor (in per man hour densities) in the study villages.
- Outdoor resting collections are done by 6 persons in 6 radials of about 0.5 Km from a central point using sweep net collections (approximately 30 sweeps per person/hour) to estimate density of outdoor resting mosquitoes in per man hour densities.
- CO2 attractant trap shown to be highly efficient for sampling *Ae. albopictus* elsewhere (Hoel et al., 2009) will be used to sample adult populations. In each area 4 traps would be setup, 500 m apart once in a fortnight. The mosquito density would be estimated in this methodology as per trap densities.
- Surveys on the breeding sources and vector breeding indices are assessed through fortnightly collections.
- DENV Infection status of field collected *Aedes* mosquitoes (eggs, immatures and adults), reared to adult stages (in the case of eggs and immatures) by RT-PCR
- Blood-meal analysis of fulfed specimens *Aedes* mosquitoes collected to understand the blood feeding habit – upto species level (DNA Barcode analysis)
- Determination of Dengue infection status of human population in the study villages by DENV IgG ELISA.
- Development and demonstration of community based IVM strategy in the area to prevent outbreaks of dengue in Kanjirappally village.

Preliminary studies were initiated in August 2013. 3 villages (Kanjirappalli, Erumeli & Koruthode) located in the fringe areas of Western Ghats in Kottayam District were selected for the study. Maximum number of Dengue cases in the District was reported from these villages. Entomological collections had been carried out (both adult and immatures) in these villages on fortnight intervals. *Ae. albopictus* was found to be the predominant species (64.86%). Immatures were kept for emergence before processing for infection. All the specimens collected (30) were processed for arbo-viral infection status and none were found infected. As suggested by the SAC, the project has been submitted for extramural funding (VSF) which has approved the project for submitting the full proposal).

1.4.5 Demonstration of mosquito vector control and prevention of vector-borne diseases through partnership and community empowerment in selected rural areas of Puducherry

IM 1304: Jan 2013 – Dec 2016

Krishnamoorthy K, Nandha B, Gunasekaran K, Hoti SL, Jambulingam P

Collaborators: *NVBDCP, Puducherry*

Introduction: Puducherry district, once highly endemic for lymphatic filariasis with about 10.3 % infection and 9.0 % disease rates (1980), is declared recently based on Transmission Assessment Survey that further rounds of

MDA can be discontinued and is free from the risk of filariasis. However, *Aedes* transmitted dengue and chikungunya are being recorded in recent times. Chikungunya was recorded in 2006 with 542 clinical cases 9 of which were positive serologically. First report of dengue was reported in 2003 and from 2011 dengue cases are reported every year and in 2012, as many as 1191 confirmed dengue cases were reported. Dengue cases were recorded from 230 locations in 11 urban Primary Health Centres (PHCs) and 146 locations in 23 rural PHCs and 2 rural Community Health Centres (CHCs). The problem has become acute particularly in rural areas where there are no organized vector control activities. Increasing trend of vector borne diseases is thus evident and integrated vector management (IVM) is appropriate, aiming at fostering collaboration and empowering communities for the prevention and control.

This study aims at developing and demonstrating a community-based IVM strategy to control/prevent vector-borne diseases through establishing collaboration and networking among various inter-sectoral partners through partnership and empowering communities to take up the responsibility and ownership of vector control towards control/prevention of vector-borne diseases. Two PHCs viz., Manadipet and Ramanathapuram with population of 7518 (7 villages) and 3615 (5 villages) were selected as intervention arm and one PHC (Thirukanur) with a population of 10528 as control arm. Five out of 12 villages in the intervention arm were recorded with seven dengue seropositive cases in 2011 out of 144 samples tested positive in Puducherry and in 2012 the incidence was about 1 per 1000. Household surveys in these villages showed that despite high levels of awareness, mosquito breeding sources were observed in about 92% of the households, with a mean number of 2.5. These observations indicate the risk of mosquito borne diseases. In 2013, during March four confirmed cases of dengue were reported from Pilayarkuppam in Ramanathapuram PHC. Entomological survey covering 493 households showed as many as 1593 breeding sources. Earthen pots and plastic drums which were used to store water constituted about 75.2%. The percentage of houses with breeding sources of *Aedes* mosquito was as high as 79.9% and the house index was 3.04%. Breeding was observed in 47.4% (Container index) of the wet containers. Breteau Index was 3.65%. Though water is supplied on all the days, the practice of storing water continues.

Based on the situation analysis different approaches for motivating the community and monitoring the community action have been identified. Schools, Self-help groups, NGOs, and Neighbourhood committees have been identified to play key role in preparing the community and mobilize their participation. Villages have been assigned with a single or combination of approaches. School students in the study area were given health education on the vector borne diseases and the methods of control. To identify the degree to which the community is prepared to take up mosquito control issue and to derive stage-specific strategies for prevention Community Readiness Model (CRM) was used to evaluate 6 dimensions using a 9 point scale using 21 anchored questions and 6 non-anchored questions. An overall readiness score for each community from the dimension scores was calculated which ranged from ranged from 3.59 to 4.23 on the 9-point scale. The mean readiness score, 3.87 corresponds with a "vauge awareness" level of readiness. Most people feel that there is a local concern, but there is no immediate motivation to do anything about it. The results suggest the need of a system for constant motivation of the community whose participation in source reduction is crucial.

Conclusion: Risk for the transmission of *Aedes* borne dengue is related to the behaviour of the community. The study is aimed to assess the feasibility of different models (approaches) towards prolonged community based action for the control of vectors and prevention of vector borne diseases.

1.5 MICROBIAL / CHEMICAL AGENTS FOR VECTOR / PARASITE CONTROL

1.5.1 Development of nanoparticle based formulation of *Bacillus thuringiensis* var. *israelensis* (VCRC B17) to improve efficacy and nanoparticles based detection system

IM 1007: Apr 2010 – Mar 2013

Manonmani AM, Hoti SL, Geetha I, Prabakaran G

Our indigenous isolate, *Bacillus thuringiensis* var. *israelensis* (VCRC B17) has been used for mosquito control. This project was undertaken to see if nano-particle based formulation would enhance the mosquito larvicidalefficacy of *Bti* so that the operational cost could be reduced and also to develop a nano-particle based method for detecting the biolarvicide (VCRC B17) in the treated sites.

Objectives:

- To develop nanoparticle based formulation of *Bti* with improved mosquito larvicidal efficacy.
- To develop nanoparticle based immunological system for the detection of *Bti* spores in the field.

Three nanoformulations of *Bti* were designed and developed. They did not show enhanced activity compared to conventional formulation when tested against the larval stages of different vector mosquito species (AR 2012).

Polyclonal *Bti* spore antibody was produced in male BALB/c mice and methods for conjugation of the antibody with gold nanoparticles standardized. When this nano-conjugated antibody was used on water and soil samples collected from mosquito breeding habitats, the detection signal of *Bacillus thuringiensis* var. *israelensis* spores from environmental samples was found but was very feeble. The efficiency of the detection system needs to be improved.

1.5.2 Development of nanotechnology based public health larvicides for effective mosquito control

IM 1006: Apr 2010 – Mar 2013

Kalyanasundaram M, Gunasekaran K

The conventional formulations of public health larvicides, water dispersible powder (WDP) and emulsifiable concentrate (EC) are used for mosquito control with limited residual activity. The current study was undertaken with an objective to prepare the nanoparticles of the public health larvicides such as temephos, pirimiphos-methyl (organophosphates) and an indigenous IGR, 2,4-Dichloro-2',6'-ditertiarybutyl diphenylether (DPE-28) to improve their larvicidal efficacy against the filariasis vector, *Cu. quinquefasciatus*.

Objectives:

- To optimize conditions for producing nanoparticles for public health larvicides by dispersion of preformed polymers by identifying a safe polymer, appropriate organic solvent and a surfactant.
- To monitor the size of the particles by Scanning Electron Microscope & Particle Size Analyzer.
- To conduct the bio-efficacy of the nanotechnology based formulation for determining LC_{50} in comparison to conventional EC formulation against *Cx. quinquefasciatus*.

The nanoparticles were made by the method, dispersion of preformed polymer for two larvicides and one IGR. Polyvinylpyrrolidone (PVP), a polymer, soluble in organic solvents was chosen as the polymer support. Dichloromethane was used as the organic solvent and sodium lauryl sulphate (SDS) as the surfactant. The conditions were optimized for the preparation of nanoparticles, which were characterized by scanning electron microscope (SEM - Hitachi S 3400N) and particle size analyzer (PSA - Zetasizerver 6.12) for the size distribution.

The characterization with SEM showed that the size of nanoparticles without the larvicide ranged from 64 to 88 nm, while the size of the nanoparticles with temephos, pirimiphos-methyl and the IGR, VCRC/DPE-28 ranged from 209 to 426 nm, 289 to 531 nm and 48 to 88 nm, respectively. The results of the PSA showed that the size distribution by intensity for nanoparticles without any larvicide was 186.1 nm, whereas for pirimiphos-methyl and DPE-28, the size distribution by intensity was 120.9 and 95.93 nm, respectively.

The nanoparticle-based and EC formulations of temephos, pirimiphos-methyl and DPE-28 were evaluated in the laboratory for their larvicidal activity against *Cx. quinquefasciatus*. The LC_{50} values of the nanoformulations of temephos, pirimiphos-methyl and DPE-28 were 87 and 73 and 771 times more than that of their respective EC formulation. Field evaluation of nanoformulation of the larvicides at 1 mg/l could produce > 90% reduction in immature density of *Cx. quinquefasciatus* in breeding habitats only for 1 – 2 days. Probably, the binding efficiency of larvicidal particles may increase with particle size from 100 nm to 10 micron as has been reported earlier in relation to drug delivery. Though, the nanoparticles are reported to play a definite role in taking the active molecules to the desired target in a closed system, especially in the drug delivery, the size of the nanoparticles does not have any positive influence on the biological activity of the larvicides especially in an open environment. Therefore, it is inferred that the use of nanoformulation for mosquito larvicides will have limited application in mosquito control.

1.5.3 Isolation and characterization of a lead molecule from the mosquito larvicidal *Euphorbia lactea* crude extract

IM 1127: Dec 2012 – Nov 2013

Samidurai K (ICMR PDF) & Nisha Mathew (Guide)

A recent work under ICMR Post doctoral fellowship led to the identification of a plant extract (*Euphorbia lactea*) with potential mosquito larvicidal activity against three species of vector mosquitoes viz., *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi*. Bioassay guided fractionation has to be done for identifying the active principle from this plant extract. This project envisages the isolation and characterization of the active principle (lead molecule) responsible for the mosquito larvicidal activity from *E. lactea* which could further be developed into a larvicidal product.

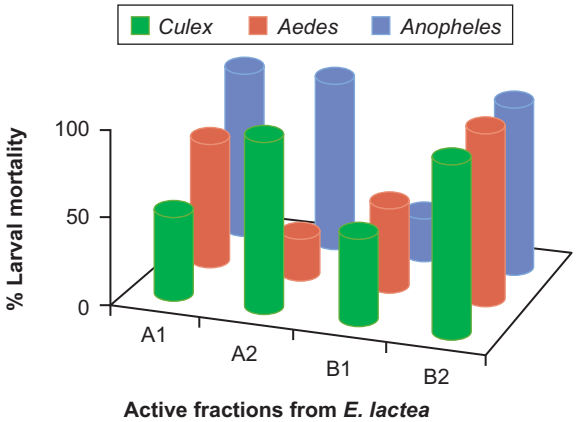
Objective:

- To isolate and characterize the mosquito larvicidal lead molecule from *Euphorbia lactea* crude extract by bioassay guided fractionation and spectral analysis.

Progress: Latex was collected from *E. lactea* plant by making incisions in the stem. The collected latex was extracted with ethyl acetate and the solvent was removed under vacuum. The residue was screened for mosquito larvicidal activity after dissolving it in ethyl alcohol/DMSO depending on the solubility. After confirming the activity the residue was subjected to chromatographic purification by using the techniques of Thin Layer Chromatography (TLC) and Column chromatography with varying composition of petroleum ether and acetone. Each fraction was collected and solvent was removed and identical fractions (by TLC) were pooled and screened for mosquito larvicidal activity at 100ppm.

The results of the bioassay guided fractionation of *E. lactea* extract are given in **FIGURE 1.5.1**. Among the fractions the A1 was effective in killing the *Culex* larvae (100% mortality) while A2 was effective against *Culex* and *Aedes* larvae. The fraction B2 was effective against all the three species of mosquito larvae tested. Fraction B2 was subjected to GC/MS analysis at IICPT, Thanjavur and identified the chemical constituents as 2,6-octadiene 2,4-dimethyl- an aliphatic hydrocarbon and 1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-,[1ar-(1aà,4á,4aá,7à,7aá,7bà)]- a tricyclic. The sesquiterpenoid lead molecule may be further used for chemical synthesis of more potent insecticidal analogues. This is the first report of the mosquitocidal lead molecule from *E. Lactea.*

FIG. 1.5.1 **Bioassay guided fractionation of *E. lactea***



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

EM 1134: Oct 2011 – Sept 2014

Poopathi S

Bacillus cereus, VCRC-B520 (NCBI: KC-119192) is a potent mosquitocidal agent isolated from marine soil and reported earlier (AR-2012). *B. cereus* was cultivated in conventional culture broth (NYSM) and used as reference medium for the present study. Chicken feather waste (CFW) collected earlier from poultry industries was washed thoroughly, shadow dried, powdered and finally stored at room temperature (30°C) till further use. The extract was made from CFW and dispensed separately into flasks for culturing mosquitocidal bacterium (*B. cereus*). The reference medium (NYSM) was also prepared along with. Both the bacterial culture media were pH adjusted (7.5) and autoclaved (at 120°C/20 lb / in^2 / 20 min). A small volume of *B. cereus* pre-culture was inoculated into all culture media and allowed to grow under orbital shaker (120 rev/min) at room temperature (30°C). Samples were drawn (2.5 ml) from each culture medium at 6 h intervals from 6 to 72 h. The culture turbidity was measured by UV-VIS spectrophotometer for analysing the bacterial growth, biomass, and toxin production. Larval bioassays were performed against mosquito species (*Cu. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*).

As the culture time increased, there was corresponding increase in the culture density, as indicated by the optical density at 650 nm. The multiplication process lasted until 72 hour. This indicated that the overall growth and production of *B. cereus* spore toxins in the culture medium was good. The biomass production was also comparable with that of the growth pattern of the bacteria. The total protein content was also estimated.

The result depicted that there was significant production of protein (toxin) and it corroborated with the results of biomass production (**FIGURE 1.5.2**). The protein profiles of *B. cereus* were estimated by SDS-PAGE (10%) (**FIGURE 1.5.3**). The result showed that the profile of toxic protein *i.e.*"Cry4Aa" (85 kDa) was distinct and conspicuous in both experimental (CFW) and conventional culture

FIG. 1.5.2 **Dynamics of mosquitocidal toxin produced from *Bacillus cereus* VCRC-B520**

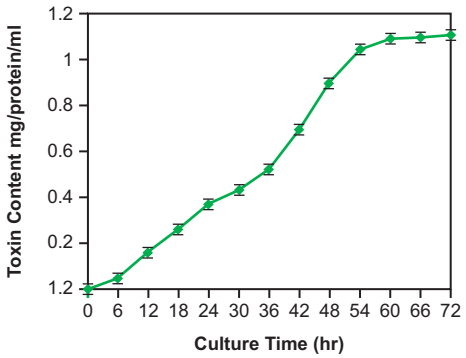
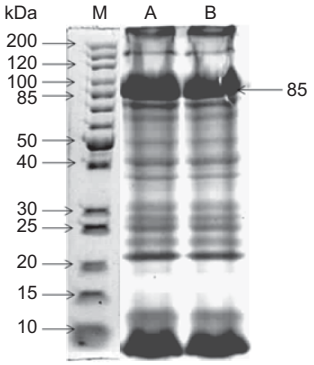


FIG. 1.5.3 **SDS-PAGE analysis of *Bacillus cereus* VCRC-B520 toxin from experimental and control media**



Lanes: A=CFW; B=NYSM; M=Protein Marker

media (NYSM). Comparative toxicity analysis of *B. cereus* from CFW and NYSM showed that the results are on par with one another as shown in **TABLE 1.5.1**.

1.5.5 Characterization of the specific polypeptide (s) in *Culex quinquefasciatus* (filariasis vector) causing resistance against biopesticides in mosquito control

EM 0503: Mar 2010 – Mar 2013

Poopathi S

Bacillus sphaericus, *Bs* is toxic to mosquito larvae and safe to non-target organism and does not pose undue risks to the environment. However, the report on the development of resistance in *Culex* species to *B. sphaericus* has impeded the success on the benefit of this bacterium in mosquito vector control operation. The object of the present project is to identify and characterize the specific polypeptide (s) in *Cu. quinquefasciatus* causing resistance against *B. sphaericus*.

Objective:

- To investigate *B. sphaericus* resistance mechanism in the filariasis vector of *Cx. quinquefasciatus*.

The development of resistance in the laboratory was given earlier (AR-2011). In the first stage of the project work, the binary toxins (42 and 51 kDa proteins) synthesized during sporulation of *Bs* were identified, purified and antibody against the toxin was raised (AR-2010).

We have characterized a protein responsible for resistance development in the filariasis vector of *Cu.*

TABLE 1.5.1 **Toxic effect of *Bacillus cereus* against mosquito species**

Bacterial Strain	Medium	Mosquito species	Intercept	Slope	LC_{50} (mg/lit)* (90% UCL-LCL) **	LC_{90} (mg/lit)* (90% UCL-LCL)	χ^2 (df)
Bacillus cereus VCRC-B520	NYSM	*Culex quinquefasciatus*	5.49	0.59 ± 0.18	0.44 (0.63 – 0.30)	3.73 (7.28 – 1.91)	6.94
		Anopheles stephensi	5.38	0.62 ± 0.17	0.54 (0.76 – 0.38)	4.19 (7.79 – 2.25)	4.27
		Aedes aegypti	4.60	0.59 ± 0.21	1.95 (2.97 – 1.28)	16.61 (44.02 – 6.26)	0.22
	CFW	*Culex quinquefasciatus*	5.47	0.64 ± 0.17	0.47 (0.67 – 0.33)	3.45 (6.48 – 1.84)	5.28
		Anopheles stephensi	5.40	0.60 ± 0.18	0.50 (0.73 – 0.35)	4.25 (8.42 – 2.14)	4.94
		Aedes aegypti	4.59	0.62 ± 0.20	1.92 (2.89 – 1.27)	15.15 (38.55 – 5.95)	0.13

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

quinquefasciatus. Laboratory selection experiments with *Bs* against the larvae were carried out up to 17 generations and the occurrence of resistance was reported (RR at LC_{50} and LC_{90} = 1987 and 2051 folds respectively). The protein profile of both resistant and susceptible larvae were qualitatively analysed by SDS-PAGE (12%) and the difference in the polypeptide pattern were analyzed. The resistant polypeptide was subsequently used for raising antibody in rabbits for acquiring anti rabbit polyclonal antibodies (IAEC/EM-0503/8/6/2010).

Immunoblotting was carried out to visualize the factor responsible for resistance. The *Bs* resistant polypeptide was subsequently eluted from the gel and the mass spectroscopic analysis (M/S-MALDI-TOF) was carried out for protein sequencing.

The inheritance of resistance based on protein profiles from mosquito population was confirmed with expression of a conspicuous polypeptide (80 kDa) in *Bs*-resistant population (**FIGURE 1.5.4**). This was again proved with western blot analysis for visualization of resistant protein. The protein was found to be reacting with anti-rabbit anti-sera (**FIGURE 1.5.5**). The result from MS analysis of peptides of resistant protein (80 kDa) was submitted to NCBI and it revealed that the resistant protein was identical 100% with "hexamerin" (XP-001843494.1), a conserved insect protein that accumulate extraordinarily in the larval stages (**FIGURE 1.5.6**). Further supportive information on the matched peptides from hexamerin (bold red colour) was mentioned in **FIGURE 1.5.7**. The data indicated that hexamerin might play a vital role on the development of resistance in *Cx. quinquefasciatus*.

FIG. 1.5.4 ***B. sphaericus*** **resistant polypeptide from** ***Cx. quinquefasciatus*** **(SDS-PAGE)**

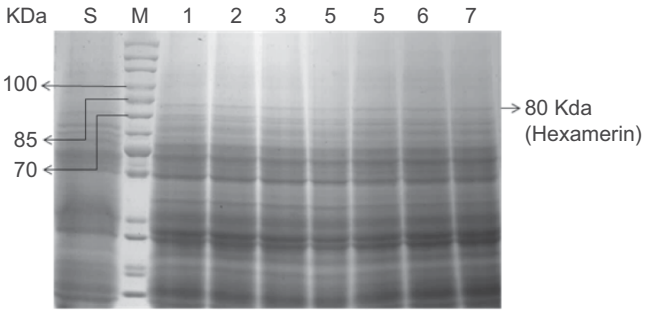


FIG. 1.5.5 **Expression of resistant protein in** ***Cx. quinquefasciatus*** **by Immunoblot analysis**

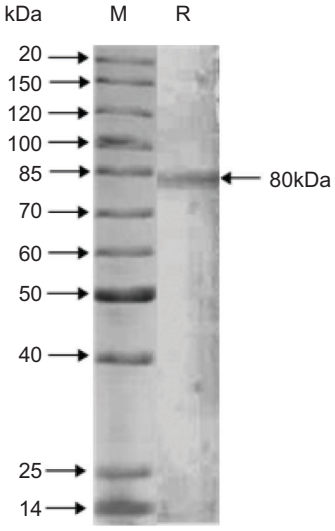
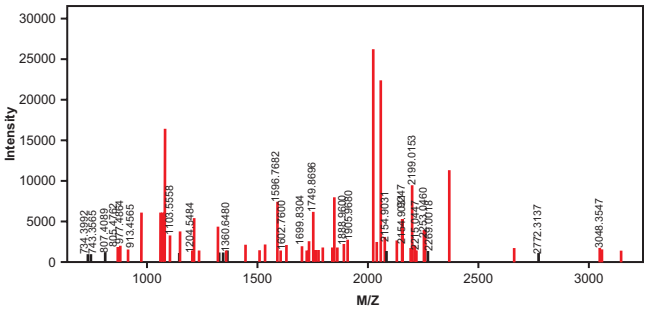


FIG. 1.5.6 **Protein mass fingerprint spectrum of** ***B. sphaericus*** **resistant polypeptide from** ***Cx. quinquefasciatus***



Note: *Number bid above illustrate that there are some peptides have been matched in the corresponding peaks. Peak strength < 5% of maximum strength are labeled black.*

FIG. 1.5.7 **Information of matched peptides from hexamerin protein, signaling the resistance development in** ***Cx. quinquefasciatus***

MTYRALVGLA ALAAGYVVVP EDKITYADKD FLVKQKQLLE VFQHVHQHEV HTELWEVSKE YKIEEHYDHY TNVEAVKEFV EFYKHGMLPF EDIFS VFHET HREQAIALFH VFYYAKDWET FYKSIVWARF HMNEGMFVY VTVAMLHRKD MAGLELPAPY EIYPYYFFNT EVIQKAAQYK MQGFYGMKKI DDVYTAIIPT NYTGWYVHTN EDQKVSYFTE DIGLNTYYYY FHADYPFWMG GKEFGLYKDR RGEMYLYKHQ QLLARYYLER LSNDLGVIPE FSWYKPIKTG YYPNMHYYNG VSFPS RDNLF NVYTPENYFE IDELIDYEHR IREVIDQGYI VLPDGSHVDL TKPESIEYLG NLIQENPDSV NTRYYKYVGE IARVLLGASV EHFDDHKVIP GALEHFETSM RDPMFYQLYK RITHWYWEFK DHLPHYTYDE INFPGLKIES AEVDKLVTYF DRYDADITNA VDVEVFDEAS MKSASTIKKF GKIAHYQGED FVIKRQWRL NHMPFTVKLN VMS EKATKGI VRMYLGPKYD AYGHVYGVNE NRENFVLLDV FPYDFVVGKN TIVRDSTKFP LYVQDRTSYF ELYKWVMDAY NGVKKFPLDM TEAHCGFPSR LM LPKGKKGG MPFQLYFIVS PYHAPSTPQY EGYDYTLNCG VGSGARYVDS LPFGYPFDRP IDEKIWFTPN M YYYDTMIYN KNEKEINAVH

Note: *Red bold words indicate corresponding peptide peaks have matched information.*

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

EM 1133: Feb 2012 – Jan 2015

Kalyanasundaram M, Paily KP

Six substituted phenyl acetyl / benzoyl piperazides were reported to exhibit moderate adulticidal activity against *Setaria digitata* under *in vitro* conditions (VCRC Annual Report, 2006). In order to identify a compound with desired macrofilaricidal activity, these compounds were tested under *in vivo* conditions against adults of lymphatic filarial parasite *Brugia malayi* (sub-periodic strain) using suitable animal models.

Objectives:

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

The ethical clearance has been obtained from Institutional Animal Ethics Committeefor use of animals.

Synthesis of base and citrate salt of promising compounds: The promising six compounds, 1-N-methyl-4 substituted phenyl acetyl piperazides (2-Chloro, 3-Chloro & 4-Chloro coded as A_4, A_5, A_6) and 1-N-methyl-4-substituted benzoyl piperazides (3-Methyl, 4-Methyl & 3,5-dimethyl coded as B_7, B_8& B_{14}) were synthesized as base and citrate salts.

Infection of animal models with Brugia malayi filarial parasite for in vivo screening: *Aedes aegypti* (Liverpool strain) mosquitoes were fed on microfilaria positive blood samples collected from *Brugia malayi* (sub-periodic strain) infected multimammate rats (*Mastomys coucha*). Infective stage parasite (L3) developed in the mosquitoes were harvested and inoculated to mongolian gerbils (*Meriones unguiculatus*) for development to adult stage. This animal model with the parasite is being maintained to harvest adult worms for transplantation and subsequent *in vivo* screening.

In vivo screening of base and citrate salt of promising compounds against B. malayi in gerbils: The method involved harvesting of adult *B. malayi* from the peritoneal cavity of mongolian gerbils and transplanting to 6 – 8 week old gerbils. After 7/8 days of transplantation with adult worms, two gerbils each were administered intraperitoneally with base and citrate salts of the compounds at 100 mg/kg b.w. consecutively for five days.

Out of the six promising compounds, five compounds, A_4, A_5,A_6, B_7 and B_8 were administered to gerbils transplanted with *B. malayi* adults at 100 mg/kg body weight. The gerbils treated with base and citrate salts of A_4, A_5 and A_6 died within 3 to 5 hours after administration on the first day, whereas the gerbils administered with base and citrate salts of B_7 and B_8 at 100 mg/kg b.w. for five consecutive days survived. Animals administered with DEC citrate and normal saline were maintained as positive and negative controls. The animals were routinely monitored for weight loss, food intake and body temperature. Animals were sacrificed 45 days after administration of the drug and the results are summarized in **TABLE 1.5.2**.

The citrate salts of B_7 and B_8 were found to exhibit 100% mortality of *B. malayi* adult worms (**FIGURE 1.5.8**) but were not effective against microfilariae. The IP administration of free base of B7 and B8 was not effective against both microfilariae and adult worms. The *in vivo* screening of free base and citrate salt of the sixth compound B_{14} is in progress.

The effective compounds (citrate salts of B_7 and B_8 and B_{14} if found effective) will be further tested in *B. malayi* infected *Mastomys coucha* through oral administration of citrate salts of the effective compounds in comparison with DEC citrate.

TABLE 1.5.2 ***In vivo* antifilarial activity of DEC analogues (Methyl substituted benzoyl piperazides) and DEC against transplanted worms of *B. malayi* in gerbils**

Compound (Dose 100mg/kg IP 5 days)	No. of animals	Average mf count in peritoneal fluid (no./20 µl)	No. of live worms transplanted (± S.D.)			No. of live worms recovered (± S.D.)			% recovery of adult worms
			Male	Female	Total	Male	Female	Total	
B7 base	2	1850	5 ± 0.71	7 ± 0.71	12 ± 1.41	2 ± 0	5 ± 0.71	7 ± 0.71	58.33
B7 citrate	2	400	5 ± 0.71	7 ± 0.71	12 ± 1.41	0	0 ± 0	0	0
B8 base	2	7625	5 ± 0.71	7 ± 0.71	12 ± 1.41	1 ± 0.71	4 ± 0	5 ± 0.71	41.67
B8 citrate	2	350	5 ± 0.71	7 ± 0.71	12 ± 1.41	0	0	0	0
DEC citrate	2	200	5 ± 0.71	7 ± 0.71	12 ± 1.41	0	0	0	0
Control	2	430	5 ± 0.71	7 ± 0.71	12 ± 1.41	4 ± 1.41	3 ± 0.71	7 ± 0.71	58.33

FIG. 1.5.8 Mortality of *B. malayi* adult worms

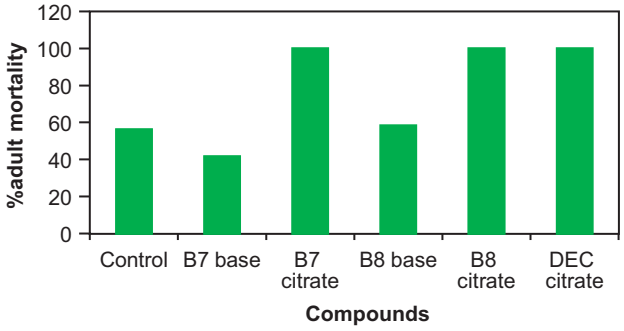
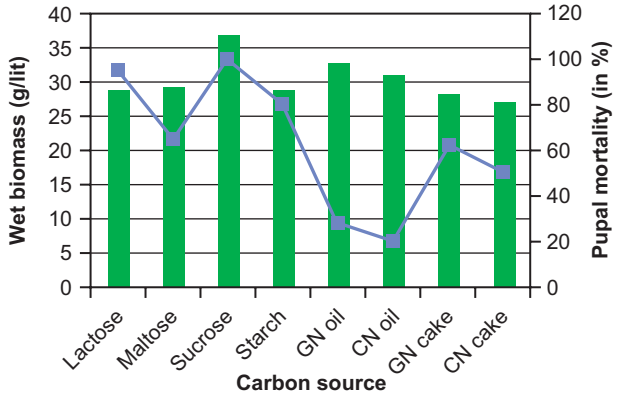


FIG. 1.5.9 Effect of different carbon sources on the growth and pupicidal activity (500 µl of CS) of VCRC B483



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

IM1302: Apr 2013 – Mar 2016

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

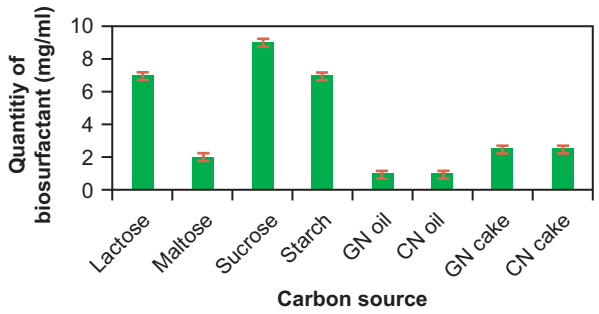
Objectives:

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

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

Upstream process for the production of the mosquitocidal metabolite(s) was optimized by designing culture medium using different carbon and nitrogen sources like lactose, maltose, sucrose, starch, groundnut oil, coconut oil,

FIG. 1.5.10 Effect of different carbon source on the production of biosurfactant by the bacterium VCRC B483



groundnut oil cake, coconut oil cake, peptone, tryptone and ammonium nitrate. Parameters studied to assess the suitable carbon source for maximum production of the metabolite was biomass, mosquitocidal activity and quantity of biosurfactant. Among the different carbon and nitrogen sources tested, sucrose and peptone were found to enhance the production of the mosquitocidal metabolite by the bacterium B483 (**FIGURES 1.5.9** & **1.5.10**). Method for the separation of the crude metabolite was also standardised. Studies on purification of the metabolite and testing the metabolites against multidrug resistant bacterial pathogens of human are in progress.

1.6 MICROBIAL / CHEMICAL AGENTS FOR VECTOR/PARASITE CONTROL UNDER TRANSLATIONAL RESEARCH

1.6.1 Development of monoterpenes extracted from the seeds of *Trachyspermum ammi* as macrofilaricidal composition

EM 1125: Jul 2011 – Jun 2013

Nisha Mathew, Paily KP, Kalyanasundaram M, Balaraman K (Consultant)

Earlier, the *in vitro* macrofilaricidal activity of *Trachyspermum ammi* extract has been reported against adult bovine filarial worm *Setaria digitata* by worm motility assay and MTT reduction assay. This project was undertaken to assess *in vitro* and *in vivo* macrofilaricidal activity of the monoterpenes and the different combinations of monoterpenes present in the fruit extract.

Objective:

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

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

During the current year, the *in vivo* screening of these combinations has been taken up. Liverpool strain of *Aedes aegypti* were allowed to feed on infected Mastomys and after 10–12 days, infective larvae (L_3) of *Brugia malayi* were collected. The L3 larvae were transmitted to Mongolian gerbils (*Meriones unguiculatus)* by intra-peritoneal inoculation of 100–200 infective larvae. Mongolian gerbils carrying 90 day old *B. malayi* infection and displaying the presence of microfilaria were used to collect adult worms for transplantation.

The results showed that when the drug is given orally in experimental animals transplanted with adult worms of *B. malayi,* the same results as observed in the *in vitro* tests could not be obtained. This may be due to the low bio-availability of the active components in the drug due to poor aqueous solubility or fast metabolism. Hence, attempts are being made to develop suitable formulations to improve the aqueous solubility and the bioavailabilty of the two promising macrofilaricidal combinations.

1.6.2 Optimization of production and downstream processing for the improved yield of Thrombinase, a blood clot dissolving enzyme, from a *Bacillus sphaericus* (strain no. NRRL B 18949)

IM 1122: Dec 2011 – Nov 2012, extension 01-12-2012 to 31-1-2013

Hoti SL, Prabakaran G and Balaraman K (Consultant)

This project relates to the improvement of a process for the production of a novel fibrinolytic enzyme, Thrombinase. The fibrinolytic enzyme is a thrombi dissolving agent having an advantageous application for the treatment of cerebral thrombosis, myocardial infarction, deep vein thrombosis and in the prevention of post-surgical adhesion. Thrombinase production process is already patented and the aim of the project is to improve the production process by varying production parameters of the fermentation, downstream processing and purification steps.

Objectives:

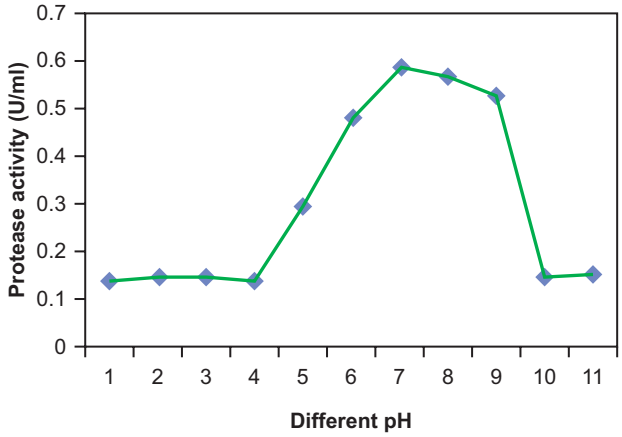
- To improve the yield of the fibrinolytic enzyme in the culture filtrate by using different media composition and changing the production parameters in the fermentor.
- To reduce the steps involved in the downstream process and purification.

Optimization of media for production of thrombinase, reduction in time involved in downstream processes and purification steps, in addition to improved yield were reported in Annual Report 2011. During last year the production was upscaled to pilot scale level. Further, the activity of Thrombinase was compared ad found to be superior to other thrombolytic agents available in market. During this year, determination of Molecular Weight of the enzyme, peptide mapping and stability of enzyme in gastric juice were studied.

Peptide mapping of Thrombinase: The HPLC purified Thrombinase was subjected to peptide analysis after digestion. The peptide profile generated yielded a total of 29 peptides of molecular weight ranging from 599 kDa to 2716 kDa and the mol weight was determined to be 28.95 kDa by MALDI-TOF.

Stability of Thrombinase: The enzyme did not show any activity at pH 2–4 indicating that the enzyme may not work at the pH equivalent to that of human gastric juice. The optimum pH for the activity of the enzyme was found to be 7.0 (**FIGURE 1.6.1**).

FIG. 1.6.1 Gastric juice stability at different PH



The improved method resulted in decrease in the number of steps and time involved in purification of Thrombinase with a marginal increase in the yield. The peptide profile generated a total of 29 peptides ranging from 599 kDa to 2716 kDa and the M.W of thrombinase was determined to be 28.95 kDa. The enzyme activity was found to be optimal at neutral pH.

1.6.3 Development of formulation and evaluation of *Pseudomonas fluorescens* (VCRC B426) against mosquito vectors

IM 1008: Jan 2010 – Dec 2012

Hoti SL, Paily KP, Prabakaran G

The culture filtrate of a bacterial isolate, *Pseudomonas fluorescens* (VCRC B426) was found to have pupicidal activity against vector mosquitoes, *Cu. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. An emulsifiable concentrate formulation of the culture filtrate caused > 80% reduction of pupal density of *Cx. quinquefasciatus* for 12 days in field conditions. However, the dosage requirement was very high for effective control of mosquito pupae in the field. Therefore, a study was undertaken with the following objectives:

Objectives:

- To scale up the production to large scale in 100 l pilot fermentor to improve the yield and finally to reduce the field dosage.
- To study different downstream process to reduce the production cost, to improve the yield and finally to reduce the field dosage.
- To develop a suitable liquid formulation.
- To test the product against different mosquito vector species, both in the lab and under field conditions.

The culture medium was modified with respect to various physico-chemical factors and it resulted in 50 fold increase in the production of the mosquito pupicidal toxin of *P. fluorescens*, when compared to the defined medium. For the recovery of maximum level of the toxin from the culture filtrate, different downstream process, viz., solvent extraction, ammonium sulphate precipitation and acid precipitation were attempted. Among the three methods, solvent extraction method yielded the maximum recovery of the pupicidal toxin from the culture broth. The results indicated that pH 7.0 and cultivation temperature 30° C are optimal for the maximum yield of the pupicidal toxin. The results of optimization of culture conditions in 100 l fermentor showed that the bacterium starts synthesizing the pupicidal toxin at 12 h (LC_{50} 555.80 µl/100 ml) and reaches the maximum at 36 h. (LC_{50} 13.82 µl/100 ml). Compared to this, in shake flask culture it took 66 h for producing the same level of toxin.

A formulation was developed and tested both in the laboratory and field conditions. Laboratory bioassay results showed that *An. stephensi* (LC_{50} 0.92 and LC_{90} 2.14 µl/100 ml) was more susceptible followed by *Ae. aegypti* (LC_{50} 4.56 and LC_{90} 6.92 µl/100 ml) and *Cx. quinquefasciatus* (LC_{50} 6.48 and LC_{90} 8.89 µl/100 ml). In a preliminary field evaluation against *Cx. quinquefasciatus*, the formulation was effective at a reduced dosage of 90 ml/m^2 resulting >90% pupal mortality up to 9 days post-treatment. Field evaluations against other species of mosquitoes such as *An. stephensi* and *Ae. aegypti* are required for assessing its potential in controlling mosquitoes breeding in different types of habitats.

1.6.4 Pilot scale production and evaluation of a mosquitocidal product based on the lipopeptides of *Bacillus subtilis*

EM 1124: Jul 2011 – Jun 2013

Manonmani AM, Geetha I and Prabakaran G

The secondary metabolites produced by a *Bacillus subtilis* subsp. *subtilis* (VCRC B471) were found to be effective on the various life stages of mosquitoes and more importantly, the pupal stages. This project was undertaken to design a cost effective production medium and up-scale the production to pilot scale level.

Objectives:

- To upscale the fermentation process for production of mosquitocidal lipopeptides of *B. subtilis*
- To optimize downstream processing techniques
- To develop formulations & testing their efficacy under laboratory conditions

A cost effective medium for the production of the mosquito pupicidal metabolite by *Bacillus subtilis* subsp. *subtilis* (VCRC B471) was designed using cheap and easily available raw materials namely, Ammonium chloride and jaggery. This medium was found to enhance the production of the metabolite by 5 times when compared to the conventional

medium. Using this medium, production was upscaled to 100 l fermentor and downstream processing techniques were optimized. Maximum production of metabolite was observed by 72 hrs (2.2g/lit). Pupicidal activity was exhibited by the 18 hour culture thereby showing that metabolite production was not associated with sporulation (AR 2011–2012). During this year seven different types of Aquoeus formulations (I –VII) were prepared and evaluated against the pupal stages of *An. stephensi*.

The LC_{50} values ranged from 2.12 to 12.7 µl for different formulations. Formulation II was found to show the maximum pupicidal activity with an LC_{50} value of 2.12 µl followed by formulation VII with an LC_{50} value of 2.6 µl. As formulation II contained both spores and metabolite, while formulation VII contained only metabolite, the latter was taken up for laboratory evaluation against important mosquito vector species and the susceptibility pattern was found to be of the following order: *An. stephensi* < *Cx. quinquefasciatus* < *Ae. aegypti* (**TABLE 1.6.1**).

This formulation needs to be tested against different mosquito species breeding in varied habitats to see if it will be an ideal candidate for use in mosquito control operations.

TABLE 1.6.1 Efficacy of formulation II against major mosquito vectors

Mosquito sp.	LC_{50} (µl/100ml)	LC_{90} (µl/100ml)
An. stephensi	2.67	9.18
Cx. quinquefasciatus	32.5	94.9
Ae. aegypti	51.1	121.1

1.7 SPONSORED

1.7.1 Small and large-scale evaluation of Natular™ T30 and G30 formulations against immatures of *Culex species* in polluted water habitats in India

EM 1210: Jul 2012 – Sep 2013

Sadanandane C, Gunasekaran K

Natular™ T30, a single layer tablet containing 8.33% spinosad and Natular™ G30, an extended release granule containing 2.5% spinosad were tested at four dosages of 25, 50, 100 and 150 mg (ai)/m^2 against *Cx. quinquefasciatus* in street drains and *Cx. tritaeniorhynchus* in abandoned wells through small-scale (phase II) and large-scale (phase III) field evaluations in Puducherry, India following WHO guidelines.

Small-scale trial: Five replicates of street drains and abandoned wells were selected for each application rate and control. Larval and pupal densities were monitored in the habitats twice a week before and after treatment. The percentage reduction of larval and pupal densities was calculated for each day of sampling; the dosage at which larval and pupal mortality were ≥80% for a longer duration was selected as the field dosage for each habitat.

Natular G30: In street drains, application of Natular G30 formulation produced ≥80% control of *Culex* immatures for one week period at 50 and 100 mg (ai)/m^2 and three weeks at 150 mg (ai)/m^2 (**FIGURE 1.7.1**). The efficacy was three times greater at 150 mg (ai)/m^2 compared to 50 and 100 mg (ai)/m^2. In abandoned wells, the G30 formulation did not give effective control at the dosages of 25 and 50 mg (ai)/m^2. At 100 mg (ai)/m^2, though, the mean reduction of pupal density was >80% on days 3–14 post-treatment, the lower limits of 95% CI for the means were <80%. Only at 150 mg (ai)/m^2, the formulation yielded ≥80% control of *Culex* immatures for 3 weeks post-treatment period (**FIGURE 1.7.2**).

Natular T30: Application of Natular T30 formulations at 25, 50, 100 and 150 mg (ai)/m^2 in street drains and abandoned wells, did not yield the desired level (≥80%) of control of *Cx quinquefasciatus* and *Cx. tritaeniorhynchus* immatures. As a result, determination of the optimum field application dosage, required for Phase III evaluation was not possible.

FIG. 1.7.1 Percent reduction of density of *Cx. quinquefasciatus* pupae in street drains treated with Natular G30

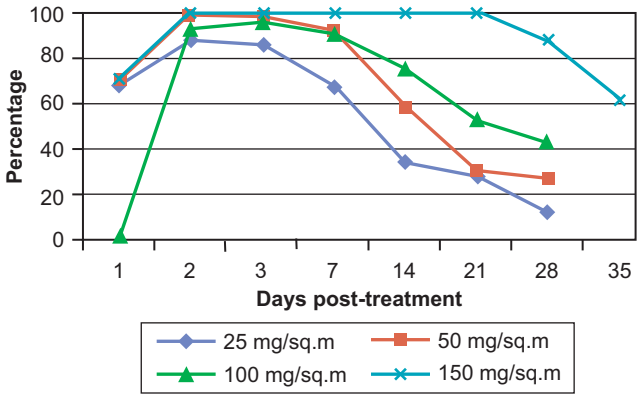
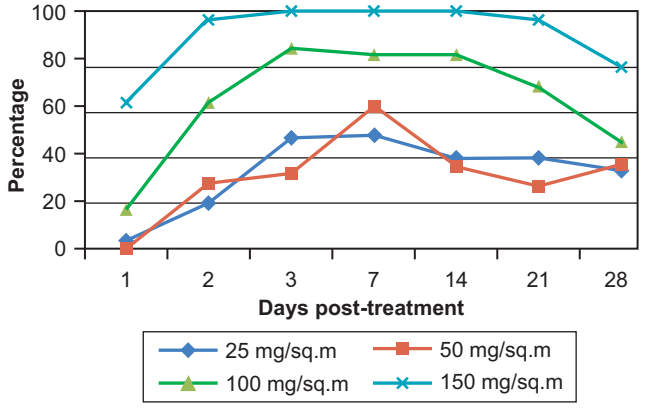


FIG. 1.7.2 **Percent reduction of density of *Cx. quinquefasciatus* pupae in abandoned wells treated with Natular G30**



Large-scale trial: Based on the small-scale trial, the dosage 150 mg (ai)/m^2 of Natular G30 was selected for the large-scale trial in street drains and abandoned wells. In street drains, the residual activity observed in large scale trial was in agreement with those of small scale trial. While in abandoned wells, increase in residual activity (≥80% reduction of pupal density for 4 weeks) was observed in large scale trial compared to small-scale trial (3 weeks). This increased residual activity might be due to less polluted water in the wells selected for phase III trial compared to the wells selected for phase II trial.

Conclusion: The trial showed that the G30 formulation of Natular could be used for larval control against *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* at the dosage of 150 mg (a.i)/m^2 at three weeks interval in street drains and at 3–4 weeks interval in abandoned wells. Natular G30 formulation could be one of the options for larval control operations in Integrated Vector Control Programmes.

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

EM 1015: 36 months (2011 – 2014)

Sahu SS, Gunasekaran K, Vijaya Kumar KN, Jambulingam P

Phase III testing and evaluation of polyester nets treated with a new LN treatment kit, ICON MAXX, (LN) in comparison with the conventionally treated nets using the same insecticide (lambda-cyhalothrin) (ITN) was continued in Koraput district, Odisha State, endemic for *Plasmodium falciparum* malaria in India.

Insecticide efficacy evaluation: Cone bioassays of *An. fluviatilis* on net (LNs) surfaces after 18 months of net distribution showed a 100% knockdown one hour post-exposure and a mortality of 100% after the holding period (24 hrs). At 24 months post-distribution of LNs, the knockdown and mortality of *An. stephensi* in the bioassays were 100% and 88.3%, respectively.

Physical inspection of nets: One year after the net distribution, all ITNs were withdrawn from the households enrolled for the study and substituted with LNs. Therefore, the surveys conducted on 18th and 24th months of net distribution were related to LNs. After 18 months of distribution, 98.2% of the LNs (n = 57) were available in the 30 households inspected, and there were holes in 17 (56.7%) out of the 30 nets randomly examined. After 24 months of distribution, 91.3% of the LNs (n = 323) were physically present in the 180 households inspected, and out of the 180 LNs examined, 45 (25%) nets were found with holes of varying sizes (**TABLE 1.7.1**).

Assessment of community acceptance and practices:

Use rate: The survey after 18 months of net distribution showed that in 93.3% of the holdings (n = 30) people were using the nets regularly and at 24 months post-distribution the net regular use rate declined to 81.1% (n = 180); occasional/seasonal use of nets (1.6%-8.9%) were also observed. In 10 (5.6%) houses, nets were not used (**TABLE 1.7.2**).

Washing practice: After 24 months of distribution, of the 180 LNs inspected, 166 (92.2%) were found washed; 13 (7.2%) were washed once, 36 (20.0%) were two or three times washed and 117 (65%) were four or more times washed. A few nets were reported to be washed even >20 times

TABLE 1.7.1 **Appearance of holes with varying sizes in LNs after 18 and 24 months of distribution**

Hole size	Survey after 18 months		Survey after 24 months	
	No. of nets with holes	Total No. of holes	No. of nets with holes	Total No. of holes
Size 1	2	4	11	32
Size 2	3	4	3	3
Size 3	2	2	7	10
Size 1 & 2	5	21	9	30
Size 1 & 3	1	4	2	6
Size 2 & 3	1	2	1	6
Size 1, 2 & 3	3	20	12	230
Total	**17**		**45**	

(**TABLE 1.7.3**). Out of the 166 washed nets, a majority (89.7%) was washed in cold water and the remainder (10.3%) in warm water. Commercially available detergent powder was used to wash the maximum number (157/166) of nets.

Adverse effect: None of the household members who slept under the LNs reported any side effect. Free from mosquito bites and no malaria was the main perceived benefits reported by the net users. The study is in progress.

TABLE 1.7.2 Usage pattern of LNs

Usage pattern	After 18 months of distribution		After 24 months of distribution	
	N + 30	%	N = 180	%
Year round-every night	28	93.3	146	81.1
Year round-occasionally	0	–	16	8.9
Seasonally-every night	0	–	5	2.8
Seasonally-occasionally	2	6.7	3	1.6
Not using	0	0	10	5.6

TABLE 1.7.3 Washing frequency of LNs after 18 and 24 months of distribution

Washing frequency	After 18 months of distribution		After 24 months of distribution	
	N = 30	%	N = 30	%
One time	13	43.3	13	7.2
Two times	9	30.0	14	7.8
Three times	4	13.3	22	12.2
Four times	1	3.3	36	20.0
Five times	1	3.3	19	10.6
Six times	0	0	20	11.1
Seven times	0	0	13	7.2
Eight times	0	0	17	9.4
10 times	0	0	5	2.8
12 times	0	0	2	1.1
15 times	0	0	1	0.6
20 times	0	0	1	0.6
24 times	0	0	2	1.1
25 times	0	0	1	0.6

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

Twelve open competition candidates, 8 under Category I and 4 under Category II (1 – Indian In-service) were admitted into the third batch of M.Sc. Public Health Entomology course affiliated to Pondicherry University.

From the first batch of students who have successfully completed their course, award of internship has been given to two students with a stipend of Rs. 12,000/ per month, based on the inter-se merit list obtained from Pondicherry University.

The 2nd PG Board of Studies meeting organized by Pondicherry University was held at VCRC on 6th May 2013 for the revision of M.Sc. PHE syllabus. The revised syllabus came into effect from the academic year 2013.

2.1.2 Post Doctoral Fellowship

One Post Doctoral Fellow is pursuing his research under the ICMR PDF programme in the Dept. of Chemistry.

2.1.3 Ph.D. Programmes

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

2.2 INDIVIDUAL STUDENTS' PROJECT

Seven students (Indian University: 3, Foreign University: 4) have undertaken projects, as detailed here under:

2.2.1 Indian University

S.No.	Name of the Student	Institution	Period (weeks)	Subject/Title
1	Ms. D. Priya B.Tech., Biotechnology	Jeppiaar Engineering College, Chennai	12	Isolation, Identification and Characterization of mosquitocidal bacteria from soil
2	Ms. K. Suganya B.Tech., Biotechnology			
3	Ms. S. Vishnu Priya B.Tech., Biotechnology			

2.2.2 Foreign University

S.No.	Name of the Student	Institution	Period (weeks)	Subject/Title
1	Ms. Celeste Franchesca Gee	Department of International Health, Georgetown University, Washington D.C, USA	15	Research Methodologies related to Vector Borne Disease Control Programs and Observation on Health System Management
2	Ms. Jenna Marie Sherry	Department of International Health, Georgetown University, Washington D.C, USA	15	Research Methodologies related to Vector Borne Disease Control Programs and Observation on Health System Management
3	Ms. Lee Vang	St. Olaf College, USA	5	Detection of Malaria using PCR and filarial infections using direct observations
4	Ms. Love Oluwafunto Odetola	St. Olaf College, USA	5	Analysis of serotypes and morbidity or mitigation of VBDs especially for dengue – Health and wellness disparities

2.3 TRAINING

Medical Officers from Sri Lanka, Research scholars and PG students from Tamil Nadu were offered training in the following areas:

S.No.	Trainee particulars	No. of Trainees	Field of Training	Period
1	Medical Officer, Anti Malaria Campaign, Colombo, Sri Lanka	1	Vector Entomology	June 2013 (1 Week)
2	Entomological Assistant, Medical Research Institute, Colombo, Sri Lanka	2	Colonization Techniques and Testing of insecticide susceptibility in medical important insects	April 2013 (1 week)
3	Ph.D. Scholar, Department of Zoology, Annamalai University, TN	3	Mosquito collection, preservation and identification	June 2013 (1 week)
4	MD Students, Dept. of Community Medicine, Pondicherry Institute of Medical Sciences, Puducherry	2	Epidemiological surveillance tool for VBDs, Control of mosquito borne diseases, Implement rapid response and disaster management w.r.t. VBD outbreaks	May 2013 (1 week)
5	MD Students, Dept. of Community Medicine, Sri Manakula Vinayagar Medical College and Hospital, Puducherry	4	Vector Control Methods	February 2013 (1 week)

2.4 VISIT

400 students from different Institutes of Tamil Nadu visited VCRC for orientation and exposure to various ongoing programmes of the centre.

S.No.	Name of the Student	Institution	No. of Students
1	Sivanthi Aditanar College, Nagercoil, Tamil Nadu	M.Sc. Microbiology	15
2	Hindustan College of Arts & Sciences, Chennai, Tamil Nadu	M.Sc. Microbiology	20
3	Pondicherry University Community College, Puducherry	Diploma in Sanitary Inspector	42
4	JIPMER, Puducherry	MBBS	30
5	Health Manpower Development Institute, Villupuram, Tamil Nadu	Multipurpose Health Worker (MHW)	50
6	Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry	B.Sc. MLT B.Sc. Nursing MHW Lady Health Visitor	40 75 32 10
7	College of Nursing, JIPMER, Puducherry	B.Sc. Nursing	73

2.5 TRAINING/WORKSHOP

S.No.	Training / Workshops	Date	No. of Participants
1	Training course for Biologists / Senior Entomologists Jointly organized by VCRC, Puducherry & NVBDCP, New Delhi	19th to 24th August, 2013	17 Biologists/Senior Entomologists (Gujarat, Haryana, Karnataka, Andhra Pradesh, Tamil Nadu & Puducherry)

2.6 NATIONAL SCIENCE DAY

National Science Day was celebrated on 28th February 2013 in T. R. Rao Auditorium, VCRC. Competitions such as Essay writing, Elocution, Pencil Sketch, Chess & Carrom were conducted for the Ph.D. Scholars and M.Sc. students. Prizes were distributed to the winners.

3 Services and Supplies

3.1 TECHNICAL SUPPORT

3.1.1 Transmission Assessment Survey in selected filariasis endemic districts

Jan 2013 – Oct 2013

In response to the request from the Directorate of National Vector Borne Diseases, a joint exercise was carried out in selected districts to plan and implement Transmission Assessment Survey (TAS) towards making decisions on stopping further rounds of Mass Drug Administration. This exercise was also intended to develop a common protocol for our country including capacity building. India is one of the 73 endemic countries for lymphatic filariasis (LF), contributing to about 50% of the 1.393 billion people who are at the risk of infection, worldwide. All the 250 endemic districts in India are covered under Annual Mass Drug Administration (MDA) with co-administration of DEC and albendazole. Having completed more than the recommended five rounds of MDA, at least 180 districts have reported less than 1% microfilaria prevalence making the areas qualified for TAS. A protocol revised and issued by the WHO was used to conduct TAS. This procedure is to determine whether a series of MDAs has successfully reduced prevalence of filarial infection below the threshold where transmission is likely no longer sustainable even in the absence of MDA, and thus MDA can be stopped.

Two districts (north & South Goa) of Goa, Thiruvarur district in Tamil Nadu and Puducherry, Karaikal and Mahe regions in Puducherry were selected by the NVBDCP for conducting TAS. All these districts (implementation units - IU) were identified as endemic based on Microfilaria (Mf) prevalence assessed by the delimitation units of National Filarias Control Programme. MDA with DEC alone was launched initially and added albendazole subsequently. More than five rounds of MDA have been completed in all these districts. The eligibility of these Implementation Units for conducting TAS was verified by compiling data using TAS eligibility reporting form. The eligibility criteria include minimum five effective (≥65% coverage) and Mf prevalence less than 1% in the entire sentinel and spot-check sites during impact assessment. All these eligibility criteria were met and thus the IUs are qualified for TAS (**TABLE 3.1.1**). A microplan for conducting TAS was prepared. The time gap between last round of MDA and the proposed TAS was more than the recommended minimum g ap of six months.

Both the IUs in Goa were combined and considered as a single evaluation unit (EU) as all the epidemiological parameters (mf rate less than 1% prior to MDA) and the implementation indicators (coverage and consumption) between the two districts (IUs) were comparable. The total population of the two districts is only 1.4 million which is less than the recommended size of 2 million for an evaluation unit. Puducherry, Karaikal and Mahe districts in Puducherry were considered as separate EUs as they are geographically separated even though the total population is less than 2 million. Thus there were five EUs subjected to TAS.

Antigenemia prevalence among young children in the age class 6-7 years is the recommended indicator to assess the impact of MDA. Positive test results in this age group are the most likely indicative of recent transmission. Immunochromatic card is the recommended diagnostic test (point of care) for antigenemia. School based survey was selected as the children enrolment in the schools was estimated to be more than 75% in all the selected evaluation units. Schools with primary section were considered as the clusters (sampling units). Enrolment was derived using the number of students enumerated in the schools and the estimated population based on census data. However, census data was found to be inflated in Mahe and data from immunization was used to estimate the children in the age class 6-5 years. As the total number of children in each of the EUs was more than 400, sample survey design was adopted. In all the EUs except Mahe, the number of clusters were more than 40, cluster based sampling was followed. In Mahe, systematic sampling method covering all the 23 schools was followed. Children from outside Mahe were also enrolled in schools in Mahe, to an extent of 40% and all of them were excluded from sampling. Survey Sample Builder tool (available at http://www.filariasis.

TABLE 3.1.1 Details on the pre-TAS criteria in the districts selected for TAS

District	Population (in Million)	Number of rounds of MDA (DEC/ DEC+albendazole	Coverage (%)	Baseline Mf prevalence (%)	Last survey Mf prevalence (%)
North Goa	0.71	5/4	93.5 – 100	0.22	0
South Goa	0.64	5/4	91.4 – 98.2	0.05	0
Thiruvarur	1.17	4/8	82.0 – 94.2	0.20	0
Puducherry	0.72	3/4	91.4 – 98.1	0.66	0
Karaikal	0.20	3/4	92.7 – 97.2	0.10	0
Mahe	0.04	3/4	91.2 – 97.1	NA	0.2

us.resources.html) was used to automate the calculations for determining the appropriate survey strategy. The inputs for the tool were the species of parasite (*Wuchereria bancrofti*) and vector (*Culex*) in the evaluation unit, the number of primary schools, total number of children in first and second standards, and average absenteeism (10-15%). The output of the exercise for all the EUs is shown in **TABLE 3.1.2**. Sampling fraction (of children within the schools) was determined based on the total children in the selected schools and the number of children to be sampled. Ten additional schools were also selected so as to achieve the required sample size in case of absenteeism. The TAS is designed to provide programme managers a critical cut-off which is the maximum number of positive ICT tests for the evaluation unit to "Pass" TAS. Training was given to all the health staff identified for the survey. Four to ten teams each with four members were involved for the ICT screening (**FIGURE 3.1.1**). The test result was read 10 minutes after loading the cards with blood sample. Tests were repeated for invalid tests resulting from the inadequate volume of blood loaded.

The number of children tested varied from 4 to 86 in different schools. The positive tests were below the critical cut-off. In Mahe all the 440 children tested with ICT showed negative reaction. In other EUs the number of children showing ICT positive ranged from 1 (Thiruvarur) to 16 (Goa). In Goa 8 clusters were found to have antigen positive children and antigen prevalence ranged between 1.8 and 25.0%. The results provided evidence for absence of transmission and hence all the EU are declared "pass". Further rounds of MDA can be stopped and post MDA surveillance has to be continued. School based survey is shown to be highly feasible as the coordination between health staff and education department was excellent. The spatial distribution of the schools selected for TAS and schools recorded with antigen positive tests in Goa is shown in **FIGURE 3.1.2**.

Transmission Assessment Survey protocol require standardization to our operational settings. Migrant children and children from other implementation units should be excluded from sampling. Data from immunization records is recommended to calculate school enrolment of children. Inflated census data was observed in some areas. The results of TAS could serve as valuable input for developing national guidelines for TAS.

FIG. 3.1.1 ICT Screening

FIG. 3.1.2 Map of Goa showing the distribution of clusters (schools) and clusters with Antigen positive children

TABLE 3.1.2 Results of TAS in the selected EUs

District	No. of school children in I & II grades	School children enrolment rate (%)	Sample size (Children 6–7 years old)	No. of clusters (schools)	No. +ve clusters	No. positive for Ag	Critical cut-off
Goa	50,982	>75	1692	47	8	16	≤20
Thiruvarur	22,718	79.2	1552	61	1	1	≤18
Puducherry	32,436	95.2	1556	30	2	2	≤18
Karaikal	6,878	92.7	1524	38	1	2	≤18
Mahe	948	97.9	439	26	0	0	≤5

UNIT 3

3.2 EPIDEMIC INVESTIGATIONS

3.2.1 Investigations on outbreak of dengue in Puducherry (2012–13)

In Puducherry, there are 24 PHCs in the rural and 10 PHCs in the urban areas. The total population is about 9,46,600 (Urban: 6,54,393; Rural: 2,92,208). First outbreak of dengue was reported in Puducherry during 2003 with 60 confirmed cases. Cases were recorded both in urban and rural areas. After a gap of 8 years, another outbreak with 185 cases was reported in 2011. In 2012 there were 1191 dengue cases. Dengue cases were recorded from 230 locations in 11 urban Primary Health Centres (PHCs) and 146 locations in 23 rural PHCs and 2 rural Community Health Centres (CHCs). Analysis of 600 cases during October and November 2012 showed that urban areas contributed about 64% of the total cases (**TABLE 3.2.1**). Incidence was about 0.63 per 1000. Incidence was significantly higher in rural areas. In Karaikal there were 51 cases and the incidence was 0.25 per 1000 population.

Relatively more number of cases have been reported among males (330) than females (270). Incidence was estimated to be 0.71 per 1000 among males compared to 0.56 among females. All the age classes were affected. There was an increase in the number of cases with age, reaching its peak in 16-20 years following which there was a gradual decline (**FIGURE 3.2.1**). Analysis of age and gender specific number of cases showed that males and individuals in the age class 16-40 years were at higher risk. It appears that places of work/study are the sites of contraction of infection.

Spatial analysis showed that almost all the PHCs were affected. Many of the areas were recorded with more than 12 cases. The number of cases and incidence however varied between the PHCs. Urban areas were more affected. Four of five areas where the incidence was above 1 per 1000 population were in the urban areas (**FIGURE 3.2.2**).

Temporal analysis showed that cases started increasing from four per day to 15 over a period of one month from October. Cases started declining thereafter. Analysis of data on the number of cases and rainfall showed that maximum number of cases was recorded during the rainy season in all the three years of outbreak. Total annual rainfall recorded for 2003, 2011 and 2012 were 1117.5 mm, 1694 mm and 370.96 mm respectively and on comparison, the year 2012 had lowest rainfall but with highest number of cases. The number of rainy days for 2003, 2011 and 2012 were 63, 98 and 50 respectively. The year 2012 is recorded with lowest precipitation despite the reporting of maximum number of dengue cases (**FIGURE 3.2.3**). These years however showed

TABLE 3.2.1 Incidence of Dengue in Puducherry during 2012

	Population	Cases	Incidence (per 1000)
Puducherry Urban	654392	383	0.59
Puducherry Rural	292208	217	0.74
Puducherry total	946600	600	0.63
Karaikal	200314	51	0.25

FIG. 3.2.1 Age and gender specific distribution of dengue cases in Puducherry during 2012

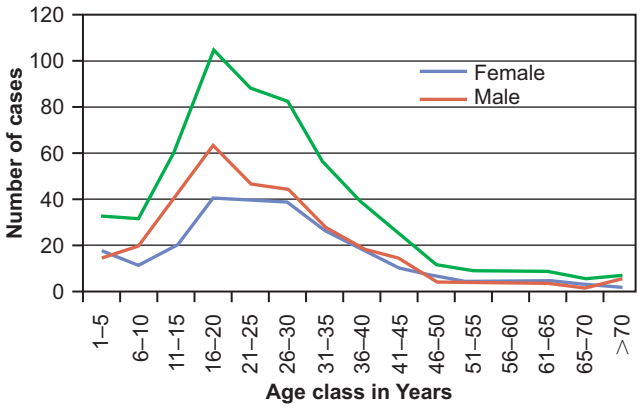
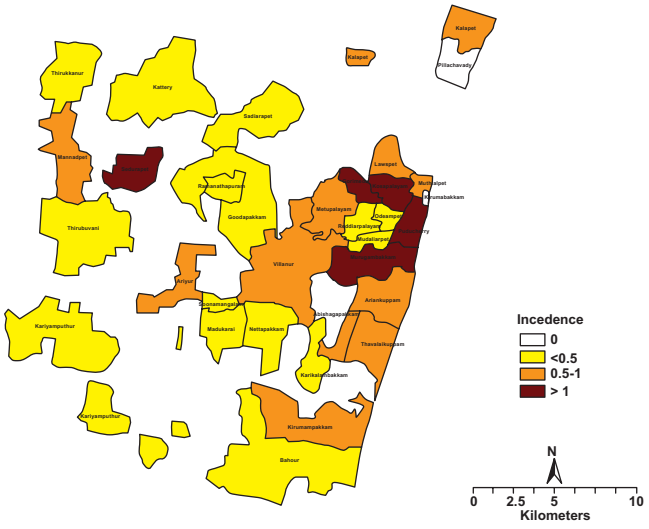


FIG. 3.2.2 Incidence (per 1000) of dengue cases in different PHCs in Puducherry



no significant variation in annual mean maximum and mean minimum temperature. Investigations carried out among 59 cases of dengue. Only three cases had the history of movement. Students and working group constitute about 32% of the cases. Employees in Govt. and private sector were 16%, while 24 and 28% constituted homemakers and others (painters, drivers, casual labourers, fishermen, business persons, etc.).

Serotypes: A total of 88 serum samples were obtained for serotyping by RT-PCR. Out of 37 samples showing positive signals for dengue viral material, 23 (62%) were serotype 3 followed by seven showing serotype 1 (19%) and five showing serotype 2 (13%). Two each (5%), with 1 & 2 and 2 & 3 mixed infections. The assays showed that three out of four dengue serotypes were prevalent during this outbreak with serotype three as the most predominant serotype. Prevalence was similar among males and females.

Distribution of serotypes in different PHCs showed that serotype three is widely distributed while others are restricted to urban areas (**FIGURE 3.2.4**).

Vector breeding and species composition: Entomological surveys were carried out in 66 and 64 areas reporting dengue cases in 2011 and 2012 respectively where a total of 11516 and 8281 containers in 574 & 654 houses were examined. House Index (HI), Container Index (CI) and Breteau Index (BI) for 2011 were 9.17, 7.36 and 14.36 respectively and these indices for 2012 were 6.57, 5.06 and 9.33 respectively. Comparison of the indices recorded in different years is shown in **TABLE 3.2.2**.

Among the water holding containers (n = 2333) examined in 2011, the predominant ones were plastic containers and plastic drums (32.75%) followed by unused vessels (29.45%) and coconut shells (13.54%). Receptacles such as tyres (2.37%), grinding stones (2.57%), flower vase (4.07%) and iron drums

FIG. 3.2.3 Dengue cases in Puducherry in 2003, 2011 and 2012 and meteorological parameters

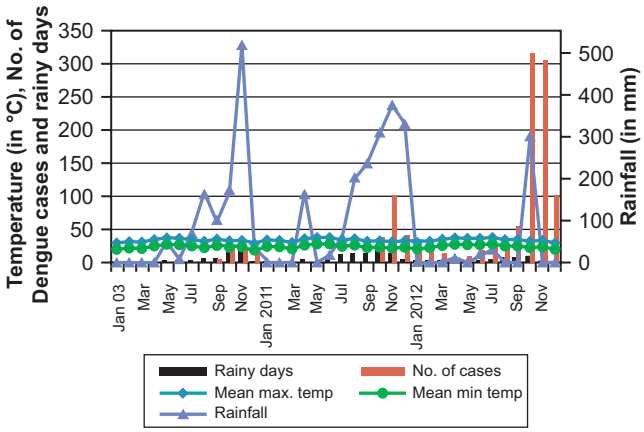


FIG. 3.2.4 Distribution of dengue serotypes in different PHCs in Puducherry

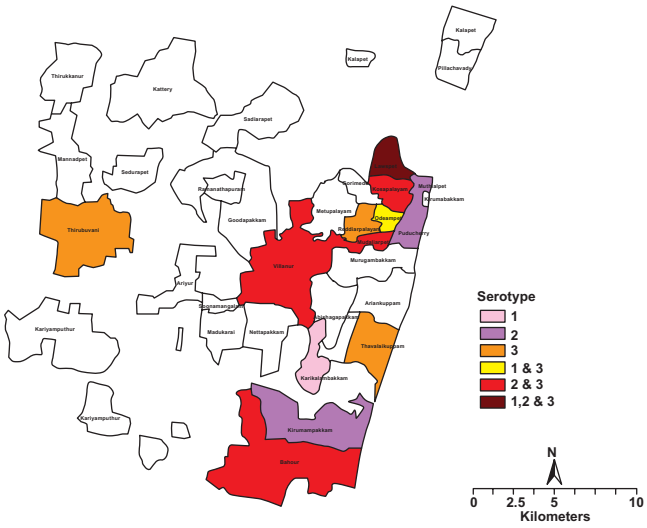


TABLE 3.2.2 Entomological indices during outbreaks of dengue in Puducherry

Parameter	Year 2003	Year 2011	Year 2012
House Index (HI)	11.33	9.16	28.97
Container Index (CI)	11.84	8.10	6.95
Breteau Index (BI)	18.77	14.36	43.18
Pupal Index (PI)	102.44	87.58	63.18
Pupae per container	6.67	0.28	0.10
Pupae per person	0.22	0.11	0.44
Species composition	*Ae. aegypti* 69.06%	*Ae. aegypti* 37.43%	*Ae. aegypti* 88.87%
	Ae. albopictus 30.94%	*Ae. albopictus* 62.57%	*Ae. albopictus* 8.81%

(2.1%), which were relatively fewer in number but their contribution in terms of pupal production was as high as 62.1%. In 2012 also plastic containers including plastic drums constituted the maximum followed by coconut shells. Unused Tyres and grinding stones were found to have higher vector breeding.

Adults emerged from immature comprised both *Ae. aegypti* and *Ae. albopictus* which were 39% and 61% respectively in 2011 (n = 11516) and for the year 2012 it was 81% and 19% (n = 9486). During the years 2003 and 2011, *Ae. albopictus* constituted about 68.9% (n = 511) and 84.8% (n = 429) of the total mosquitoes emerged from the immature samples. In urban areas, it was 24.58% (n = 1087) and 47.20% (n = 345) respectively for 2003 and 2011. During 2012, *Ae. aegypti* was predominant both in rural and urban areas constituting 85.32% and 71.83% respectively out of total mosquitoes emerged from the sample. This indicates that *Ae. aegypti* has preponderance over *Ae. albopictus* over period of time. Vector survey was carried out in the residential areas reporting dengue cases. A total of 468 houses were inspected to assess the breeding indices, pupal productivity and species composition. In these houses, a total of 7289 containers were examined for *Aedes* breeding. The percent of wet containers was low (17.65%) and House Index (HI), Container Index (CI) and Breteau Index (BI) was 7.27, 3.8 and 10.47 respectively. The pupal index and pupae per container values were 6.41 and 0.02 respectively.

During 2012, 64 sites were surveyed for vector breeding. *Ae. aegypti* breeding was recorded from 21 sites while *Ae. albopictus* was recorded from 11 sites. Both the species were found to co-exist in 11 sites. *Ae. aegypti* was the predominant species. Recommendations were given for epidemic preparedness and response based on the risk factors. Also, suggested establishing Epidemic Control Committee, preparation of site specific epidemic preparedness and response plans, strengthening epidemiological surveillance and social mobilization for sustained preventive measures.

3.3 FACILITIES

3.3.1 Laboratory animal house

The laboratory animal facility of the Centre is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and has breeding colonies of animals such as BALB/c mice (*Mus musculus*), mongolian gerbils (*Meriones unguiculatus*), and multimammate rats (*Mastomys coucha*). Animals of these species are being used for various ongoing research projects of the Centre. Experiments using animals are conducted after getting the approval of the Institutional Animal Ethics Committee (IAEC) and during the reporting period, the meeting of the committee was held on 21st June 2013. Progress of eight ongoing projects was reviewed by the committee and three new projects were approved for use of animals for experiments. Health status of the animals is being monitored by a visiting veterinary doctor.

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

The *Brugia malayi* (sub-periodic strain) filarial parasite is maintained in the animal models, mongolian gerbil (*Meriones unguiculatus*) and multimammate rat (*Mastomys coucha)*. Early stages of the parasite (L1 to L3) are developed in *Ae. aegypti* (Liverpool strain) by feeding them on microfilaraemic blood and the infective larvae (L3) obtained are inoculated to the animal models for development and patent infection with microfilariae. As on date, there are 16 multimammate rats and 8 gerbils inoculated with the L3 and on an average 70% of the animals are developing the parasite to patent infection. Adults and mf collected from these animals are used for various projects on development of stage specific diagnostics and drug screening related to bancroftian filariasis.

3.3.3 Microbial Culture Collection

The Microbial Culture Collection (MCC), available at the Unit of Microbiology and Molecular Biology functions as a depository to supply microbial cultures to the scientists/scholars working on mosquitocidal bacterial pesticides. Presently, the MCC has two sections, the Bacteria and Fungi collectively holding over 700 cultures. Generally cultures are preserved at –80°C and also by freeze-drying. Preserved microbial agents are periodically checked for viability and some key characteristics so that cultures continue to represent the original deposits. A dozen of these agents have been patented in view of their mosquitocidal activity or pharmacological importance. These patented bacterial and fungal strains are checked at bimonthly intervals.

3.3.4 Rearing and colonization of mosquitoes

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

Mosquitoes (Diptera: Culicidae)

1. *Culex quinquefasciatus*
2. *Anopheles stephensi*
3. *Aedes aegypti*
4. *Toxorhynchites splendens*

In addition, two species of larvivorous fish are maintained in the laboratory.

1. *Gambusia affinis*
2. *Poecilia reticulata*

Immatures and adult stages of mosquitoes were supplied to various divisions of the Centre for carrying out basic studies on biology and susceptibility to insecticides and efficacy evaluation of biocides and other vector control products. Also, mosquito samples were provided to the Illustration Division of the Centre for organizing exhibitions/demonstrations in Schools, colleges and Primary Health centres and at community gatherings to create awareness on vector borne diseases, mosquito life stages and their control (**TABLE 3.3.1**).

TABLE 3.3.1 Supplies from Rearing & Colonization Division to different laboratories during 2013

Species	Internal (within VCRC)				External*		Total
	Vector Biology & Control	Microbiology, Immunology & Bioinformatics	Human Resource Development	Chemistry	Schools & Colleges	NVBDCP, Ariyur PHC, Puducherry	
Culex quinquefasciatus							
Immature stages	–	306040	12775	22590	1500	1000	343905
Adults	–	9150	3115	–	300	200	12765
Anopheles stephensi							
Immature stages	–	108615	9725	21540	1500	1000	142380
Adults	20100	1100	1850	–	300	200	23550
Aedes aegypti							
Immature stages	7400	56585	9100	23590	1500	1000	99175
Adults	–	325	–	–	300	200	825

** Supplies were provided for Exhibition purpose*

4 Publications

1. Kalyanasundaram M, Gunasekaran K. Synthesis, characterization and evaluation of nanoparticles of public health larvicides for mosquito control. Journal of Vector Borne Diseases 2013; 50:1–4.
2. Karunamoorthi K, Sabesan S. Insecticide Resistance in Insect Vectors of Disease with Special Reference to Mosquitoes: A Potential Threat to Global Public Health. Health Scope 2013; 2(1):4–18.
3. Karunamoorthi K, Sabesan S, Jegajeevanram K, Vijayalakshmi J. Role of traditional antimalarial plants in the battle against the global malaria burden. Vector Borne Zoonotic Diseases 2013; 13(8):521–44.
4. Manonmani AM, Mathivanan A, Sadanandane C, Jambulingam P. Evaluation of the mtDNA-COII region based species specific assay for identifying members of the *Anopheles culicifacies* species complex. Journal of Arthropod-Borne Diseases 2013; 7(2):154–63.
5. Nandha B, Krishnamoorthy K, Jambulingam P. Towards elimination of lymphatic filariasis: social mobilization issues and challenges in mass drug administration with anti-filarial drugs in Tamil Nadu, South India. Health Education Research 2013; 28(4):591–8.
6. Nisha M, Elango A, Sabesan S, Kalyanasundaram M. Mosquito attractant blends to trap host seeking *Aedes aegypti*. Parasitology Research 2013; 112(3):1305–12.
7. Paily KP, Chandhiran K, Vanamail P, Pradeep Kumar N, Jambulingam P. Efficacy of a mermithid nematode *Romanomermis iyengari* (Welch) (Nematoda: Mermithidae) in controlling tree hole-breeding mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) in a rubber plantation area of Kerala, India. Parasitology Research 2013; 112(3):1299–304.
8. Poopathi S, Archana B. Management of waste product from watermelon for culture and production of mosquitocidal toxins (bio-pesticide). International Journal of Environment and Waste Management 2013; 12(4):442–52.
9. Poopathi S, Mani C, Rajeswari G. Potential of sugarcane bagasse (agro-industrial waste) for the production of *Bacillus thuringiensis israelensis*. Tropical Biomedicine 2013; 30 (3):504–15.
10. Das LK, Hari Chandra Kumar KT, Vijayalakshmi G, De Britto LJ. Effect of domiciliary limb hygiene alone on lymphodema volume and locomotor function in filarial lymphodema patients in Puducherry, India. Journal of Communicable Diseases 2013; 45(1-2):17-23.
11. Poopathi S, Mani C, Vignesh V, Praba VL, Thirugnanasambantham K. Genotypic diversity of mosquitocidal bacteria (*Bacillus sphaericus, B. thuringiensis,* and *B. cereus*) newly isolated from natural sources. Applied Biochemistry and Biotechnology 2013; 171: 2233–46.
12. Pradeep Kumar N, Jayakumar PR, George K, Kamaraj T, Krishnamoorthy K, Sabesan S, et al. Genetic characterization of Dengue viruses prevalent in Kerala state, India. Journal of Medical Microbiology 2013; 62(4):545–52.
13. Pradeep Kumar N, Krishnamoorthy N, Sahu SS, Rajavel AR, Sabesan S, Jambulingam P. DNA Barcodes indicate members of the *Anopheles fluviatilis* (Diptera: Culicidae) species complex to be conspecific in India. Molecular Ecology Resource 2013; 13(3):354–61.
14. Sabesan S, Raju KH, Subramanian S, Srivastava PK, Jambulingam P. Lymphatic Filariasis Transmission Risk Map of India, Based on a Geo-Environmental Risk Model. Vector Borne Zoonotic Diseases 2013; 13(9):657–65.
15. Sahu SS, Gunasekaran K, Vanamail P, Jambulingam P. Persistent foci of falciparum malaria among tribes over two decades in Koraput district of Odisha State, India. Malar Journal 2013; 12(1):72.
16. Samidurai K, Nisha M. Mosquito larvicidal and ovicidal activity of puffer fish extracts against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Tropical Biomedicine 2013; 30(1):27–35.
17. Sankari T, Hoti SL, Das LK, Govindaraj V, Das PK. Effect of Diethylcarbamazine (DEC) on prostaglandin levels in *Wuchereria bancrofti* infected microfilaraemics. Parasitology Research 2013; 112(6):2353–9.
18. Sharma R, Hoti SL, Vasuki V, Sankari T, Meena RL, Das PK. Filamentation temperature-sensitive protein Z (FtsZ) of *Wolbachia*, endosymbiont of *Wuchereria bancrofti*: a potential target for anti-filarial chemotherapy. Acta Tropica 2013; 125(3):330–8.

19. Srinivasan R, Jambulingam P. *Sergentomyia* (Parrotomyia) *jerighatiansis*, a new species of sand fly (Diptera: Psychodidae: Phlebotominae) from Kandhamal district, Orissa, India. Acta Tropica 2013; 128:674–79.
20. Srinivasan R, Jambulingam P, Vanamail P. Sand Fly (Diptera: Psychodidae) Abundance and Species Diversity in Relation to Environmental Factors in Parts of Coastal Plains of Southern India. Journal of Medical Entomology 2013; 50(4):758–63.
21. Suhail J and Sabesan S. *Aedes* vector population dynamics and occurrence of dengue fever in relation to climate variables in Puducherry, South India. International Journal of Current Microbiology and Applied Sciences 2013; 12(2):313–22
22. Twinkle K, Nisha M, Lakshmy S, Kalyanasundaram M. Synthesis and Macrofilaricidal Activity of Substituted 2-Hydroxy/5-Hydroxy/2-Methyl-1, 4-Naphthoquinones. Drug Development Research 2013; 74:216–26.
23. Vasuki V, Hoti SL, Kamaraj N, Ghosh S, Rajaram K. Optimisation of an asymmetric polymerase chain reaction assay for the amplification of single-stranded DNA from *Wuchereria bancrofti* for electrochemical detection. Mem Inst Oswaldo Cruz 2013; 108(6):804–7.

Accepted

24. Geetha I, Aruna R, Manonmani AM. Mosquitocidal *Bacillus amyloliquefaciens*: Dynamics of growth and production of novel pupicidal biosurfactant. Indian Journal of Medical Research (in press) 2013.
25. Gunasekaran K, Sahu SS, Vijayakumar T, Subramanian S, Jambulingam P. Effect of house spraying with lambdacyhalothrin 10% CS formulation in comparison to 10% WP against malaria vector in Malkangiri District, Odisha, India. Indian Journal of Medical Research (in press) 2013.
26. Gunasekaran K, Sahu SS, Jambulingam P. Estimation of vectorial capacity of *Anopheles* minimus Theobald and *Anopheles fluviatilis* James (Diptera: Culicidae) in a malaria endemic area of Odisha State, India. Indian Journal of Medical Research (in press) 2013.
27. Poopathi S, Ahangar NA, Lakshmi Praba V, Mani C. Isolation and Characterization of a new mosquitocidal bacterium (*Enterobacter cloacae* VCRC-B519) from marine soil. Biocontrol Science and Technology (in press) 2013.
28. Poopathi S, Thirugnanasambantham K, Ragul K. Purification and characterization of keratinase from feather degrading bacterium useful for mosquito control - A new report. Tropical BioMedicine (in press) 2013.
29. Sahu SS, Gunasekaran K, Raju HK, Vanamail P, Pradhan MM, Jambulingam P. Response of the malaria vectors to the conventional insecticides in the southern districts of Odisha State, India. Indian Journal of Medical Research (in press) 2013.
30. Sahu SS, Gunasekaran K, Jambulingam P. Environmental management through sluice gated bed-dam: a revived strategy for the control of *Anopheles fluviatilis* breeding in streams. Indian Journal of Medical Research (in press) 2013.
31. Shriram AN, Krishnamoorthy K, Vanamail P. Survival of diurnally sub periodic *Wuchereria bancrofti* parasite in *Downsiomyianivea* (Diptera: Culicidae): a density dependent factor from Andaman & Nicobar Islands. Indian Journal of Medical Research (in press) 2013.

5 Meetings / Seminars/ Symposia / Conferences / Workshops / Guest Lectures Delivered

5.1 MEETINGS/SEMINARS/WORKSHOPS ATTENDED BY THE SCIENTISTS (2013)

Date	Particulars	Scientist
4 – 6 Jan	Attended the meeting at Malkangiri with Dr. B. R Thapper, JE Consultant, NVBDCP Delhi, Dr. M. M. Pradhan, Deputy Director, NVBDCP, Odisha and CDMO, Malkangiri regarding suspected JE outbreak at Malkangiri	Dr. S.S. Sahu
11 – 12 Jan	High Powered Committee(HPC) Meeting to evaluate the ongoing activities of ICMR institutes working in the area of vector borne diseases and its control held at ICMR Hqrs., New Delhi	Dr. P. Jambulingam
21 Jan	Meeting to review HPC document on LF at ICMR HQ	Dr. K. Krishnamoorthy
29 Jan	Resource person to train District Malaria Officers/ District Entomologists/Senior Entomologists, Department of Public Health and Preventive Medicine, Govt. of Tamil Nadu on transmission assessment survey and elimination of filariasis	Dr. K. Krishnamoorthy
30 Jan	Attended the meeting of Board of Studies in Chemistry in the Department of Chemistry, KMCPGS, Lawspet, Puducherry, to frame the curriculum of M.Sc., & M.Phil. Courses under CBCS sytem to be implemented from 2013–14	Dr. M. Kalyanasundaram
1 Feb	Expert Member in the Selection Committee Meeting (JRF - UGC) at KMC for PG Studies, Puducherry	Dr. S. Sabesan
4 Feb	External Expert for Curriculum Committee meeting of PG course, Dept. of Zoology, KMC for PG Studies, Puducherry	Dr. S. Sabesan
13 – 17 Feb	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Goa	Dr. K. Krishnamoorthy Dr. S. Subramanian
18 – 22 Feb	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Thiurvarur	Dr. K. Krishnamoorthy
19 – 20 Feb	Temporary adviser to RD for the “informal inter-country consultation to intensify malaria control towards elimination” held at WHO-SEARO, Delhi	Dr. P. Jambulingam
28 Feb	UGC Sponsored guest lecture on “Public Health and Mosquitoes” at Post Graduate and Research Department of Zoology & Wildlife Biology, AVC College (Autonomous), Mayiladuturai, Tamilnadu	Dr. K. Gunasekaran
4 – 5 Mar	Meeting of the Technical Working Group on LF Entomology Handbook organized by CNTD, WHO, Geneva held at Liverpool, UK	Dr. P. Jambulingam
9 – 10 Mar	Attended workshop on “Efficient, Quality-Assured Data Capture using EpiData” Organized by International Union Against Tuberculosis and Lung Disease at JIPMER, Puducherry, India	Dr. R. Srinivasan Dr. C. Sadanandane
11 – 15 Mar	Regional Workshop on Dengue Vector Management held at WHO SEARO, Colombo, Sri Lanka	Dr. P. Jambulingam

Date	Particulars	Scientist
16 Mar	Invited as a resource person to deliver a guest lecture on "Natural Product screening towards drug discovery" at the Multipurpose Hall of MTPG & RIHS, Puducherry in the National Conference on "Recent Trends in Industrial Pharmacognozy"	Dr. M. Kalyanasundaram
18 Mar	TAS planning meeting at Directorate of Public Health and Preventive Medicine, Chennai	Dr. K. Krishnamoorthy
25 – 26 Mar	Participated in the Workshop on Executive Development Programme for College Principals conducted at Pondicherry University on 25th & 26th March 2013	Dr. A.M. Manonmani
1 – 3 Apr	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Puducherry	Dr. K. Krishnamoorthy Dr. S. Subramanian
3 Apr	Attended the Workshop on Bioethics for Members of Institutional Ethics Committee organized by the Division of Research, JIPMER, Puducherry	Dr. M. Kalyanasundaram
3 Apr	Attended a workshop on "Bioethics for Members of Institute Bioethics Committee" conducted by JIPMER at JIPMER	Dr. L.K. Das
3 Apr	Attended the meeting with the Joint Director of Malaria, Dr. V. B. N. Rao, Chief Entomologist, Govt. of Odisha at CDMO office, Koraput to review the malaria situation in Koraput district in the context of distribution of 2.5 lakhs LLINs in the district	Dr. S.S. Sahu
12 – 13 Apr	Chaired a session on `Drugs & Pharmaceuticals – Research & Development' during the `National Workshop & Industry – Academia Interaction Meet' held at Pondicherry University	Dr. A.M. Manonmani
15 Apr	ICMR's 8th Tribal Health Research Forum Meeting at VCRC, Puducherry	Dr. K. Gunasekaran Dr. S. Subramanian Dr. L.K. Das
25 Apr	Attended the meeting to observe the 'Observation of Malaria Day' at CDMO conference Hall at Koraput under the chairmanship of the Collector and District Magistrate, Koraput	Dr. S.S. Sahu
25 Apr	Guest lecture on 'Malaria and Vector Control' at Aarupadai Veedu Medical College & Hospital, Kirumampakkam, Puducherry in connection with their celebration of World Malaria Day	Dr. K. Gunasekaran
27 – 29 Apr	Attended 24th National Congress of Parasitology and presented a paper on oral mode titled "Development of an antibody assay to measure the exposure rate of endemic residents to filarial infection" at Regional Medical Research Centre for Tribals, Jabalpur, Madhya Pradesh	Dr. K. Athisayamary
29 Apr	ICMR Meeting on finalization of the SOP for introduction of public health pesticides including biolarvicides in the National Control Programme organized at NIOP, Delhi	Dr. K. Gunasekaran
6 May	Attended the meeting of the PG Board of Studies in Medical Entomology to revise the Syllabi and Regulations of M.Sc. Public Health Entomology at 10 30 A M at the Council Hall, Vector Control Research Centre, Puducherry	Dr. M. Kalyanasundaram Dr. K. Gunasekaran Dr. A.M. Manonmani
8 May	ICMR Expert Group Meeting on "Insecticides" at NIMR	Dr. K. Gunasekaran
10 May	ICMR Task Force Meeting on "Insecticide Resistance" organized at NIMR	Dr. K. Gunasekaran
14 – 16 May	Malaria Regional Technical Advisory Group Meeting held at WHO SEARO, New Delhi	Dr. P. Jambulingam
3 – 7 June	Imparted field training on Entomological and Parasitological aspects of Malaria and Control in Odisha state to a trainee from Sri Lanka sponsored by WHO	Dr. S.S. Sahu
10 Jun	Internship training on animal taxonomy held at Zoological Survey of India (ZSI), Chennai Inaugural address & delivered a talk on "Insect vectors & Human health"	Dr. P. Jambulingam
18 – 19 Jun	National Workshop on Transmission Assessment Survey (TAS) using ICT cards for Elimination of Lymphatic Filariasis in India held at Pune	Dr. K. Krishnamoorthy

Date	Particulars	Scientist
25 – 31 Jun	Regional Programme Review Group meeting at Dili, East Timor	Dr. K. Krishnamoorthy
5 Jul	Attended a Task Force meeting on Dengue preparedness at CDMO Conference Hall, Koraput district, Odisha organized by the district Health Department, Koraput	Dr. S.S. Sahu
9 Jul	Attended the 55th meeting on Town Official Language at JIPMER, Puducherry	Dr. L.K. Das
11 Jul	State Level Task Force Meeting on LF elimination programme in Tamil Nadu, at the Secretariat, Ministry of Health, Govt. of Tamil Nadu	Dr. K. Krishnamoorthy Dr. S. Subramanian
11 Jul	First meeting of the 'Task force to combat mosquito menace' organized by the Dept. of Local Administration, Govt. of Puducherry at the Secretariat, Govt. of Puducherry	Dr. K. Gunasekaran
12 Jul	Delivered a lecture on 'Sensitization to control Dengue, Malaria and Diarrhoea' at Jeypore Municipality Hall at Jeypore organized by district heath department, Koraput	Dr. S.S. Sahu
17 Jul	Invited as an External expert member for the equipment specification vetting meeting, Medicine Department, JIPMER, Puducherry	Dr. Nisha Mathew
25 – 31 Jul	Elimination of LF in Nan cowry group of islands – stakeholders meeting for finalizing implementation plan at RMRC, Port Blair	Dr. K. Krishnamoorthy
29 Jul	Attended a Workshop on 'Organisation-cum- Sensitization and Monitoring on MDD Campaign-2013' at CDMO Conference Hall, Koraput district, Odisha organized by the Zilla Swasthya Samiti and NVBDCP, Koraput	Dr. S.S. Sahu
2 – 3 Aug	Training State level Health Inspectors in conducting Sample Blood survey evaluate LF elimination programme in Mahe	Dr. K. Krishnamoorthy
9 – 10 Aug	Tribal health forum meeting at DMRC, Jodhpur	Dr. K. Gunasekaran Dr. L.K. Das
9 – 25 Aug	Biologists and Senior Entomologists, NVBDCP training	Dr. K. Krishnamoorthy Dr. S. Subramanian
28 Aug	Invited speaker to deliver a guest lecture on "PCR optimization and troubleshooting" at the Tenth National Workshop on "Basic Techniques in Molecular Biology & Bioinformatics in Pharmacogenomics" at the Department of Pharmacology, JIPMER	Dr. V. Vasuki
28 Aug	Live Tele-bridge programme on "Beware of mosquitoes" organized by the Doordarshan Kendra, Puducherry	Dr. K. Gunasekaran
30 Aug	Invited as a Resource Person to deliver a special lecture on "Target Based Drug Discovery" in ICMR sponsored National Seminar on Emerging Trends in Drug Discovery at the Multipurpose Hall of MTPG&RIHS, Puducherry	Dr. M. Kalyanasundaram
5 Sept	"INDIAPEST-2013" organized by the Indian Pest Control Association at the Park-Hyderabad, Participated and delivered a guest lecture on "Mosquito control in different structures"	Dr. K. Gunasekaran
9 Sept – 11 Oct	Advanced Techno-Management" programme for the scientists of D,E,F cadres organized by Administrative Staff College of India Hyderabad	Dr. R.L.J. De Britto
12 – 13 Sept	National Workshop on Transmission Assessment Survey (TAS) using ICT cards for Elimination of Lymphatic Filariasis in India held at Bhubaneshwar	Dr. K. Krishnamoorthy
15 – 17 Sept	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Diu	Dr. K. Krishnamoorthy
16 – 18 Sept	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Karaikal	Dr. K. Krishnamoorthy Dr. S. Subramanian
24 Sept	Participated Stakeholders' Consultation Workshop Presenting State Action Plan on Climate Change, Puducehrry organized by DSTE & Project Implemntation Agency, Govt. of Puducherry	Dr. S. Sabesan

Date	Particulars	Scientist
25 – 26 Sept	National Workshop on Transmission Assessment Survey (TAS) using ICT cards for Elimination of Lymphatic Filariasis in India held at Chennai	Dr. K. Krishnamoorthy
30 Sept – 1 Oct	National Workshop on Transmission Assessment Survey (TAS) using ICT cards for Elimination of Lymphatic Filariasis in India held at Bangalore	Dr. K. Krishnamoorthy
12 – 14 Oct	Training State level Health Inspectors in Transmission Assessment Survey (TAS) in and provided technical support for conducting TAS in evaluating LF elimination programme in Mahe	Dr. K. Krishnamoorthy
17 –19 Oct	2nd National Knowledge Network Annual Workshop	Dr. S. Subramanian

6 Institutional Committees

6.1 EXTERNAL COMMITTEES

Scientific Advisory Committee Members of 35th SAC meeting of VCRC

Prof. M.K.K. Pillai
Chairman
Prof. & Head, Dept. of Zoology (Retd.)
Delhi University
47, Anubam Appartments,
B-13, Vasundhara Enclave,
Delhi – 110 096

Dr. S. Adiga
Member
Former Director NNRMS-RRSSC
ISRO, Bangalore
Advisor, RSI Softech India Pvt. Ltd.
1st floor, Elegant Residency II,
4, I Cross, ISRO Layout,
Bangalore – 560 078

Prof. (Dr.) Ashok Kumar Das
Member
Senior Professor & Head
Department of Endocrinology,
JIPMER
Puducherry – 605 006

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

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. S.K. Puri
Member
Scientist G & Scientist-in-Charge
Parasitology Division
Central Drug Research Institute,
Lucknow – 226 001

Padmashri Dr. P.K. Rajagopalan
Member
Former Director, VCRC
2E, Ramaniyam, Lakshmi Apartments,
29, I Seaward Road, Valmiki Nagar,
Chennai – 600 041

Dr. Rashmi Arora
ICMR Representative
Chief, ECD Division
Indian Council of Medical Research
Ansari Nagar,
New Delhi – 110 029

Dr. Sarala K. Subbarao
DG's Nominee & VBDSF Expert
INSA Sr. Scientist
Former Director, NIMR
Indian Council of Medical Research
Ansari Nagar,
New Delhi – 110 029

Dr. A.P. Dash
VBDSF Expert
Former Regional Adviser (VBDC)
WHO-SEARO
Flat No. 112 FF, Milano Mahagun Mansion II,
Plot No. 1/4 Vaibhavkhand, Indrapuram,
Ghaziabad – 201 014, Uttar Pradesh

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

Dr. Shiv Lal
VBDSF Expert
Former Special DG, DGHS & Former Director, NCDC
Programme Coordinator cum Adviser - JE/AES
National Vector Borne Disease Control Programme
22, Sham Nath Marg,
New Delhi – 110 054

Dr. P.K. Srivastava
VBDSF Expert
Joint Director
National Vector Borne Disease Control Programme
22, Sham Nath Marg,
New Delhi – 110 054

Dr. S.K. Kar
Statutory Member
Director
Regional Medical Research Centre
Chandrasekharpur,
Nandankanan Road,
Bhubaneswar – 751 016, Odisha

Dr. J. Mahanta
Statutory Member
Director
Regional Medical Research Centre
Post Box No.105,
Dibrugarh – 786 001, Assam

Dr. D.T. Mourya
Statutory Member
Director
National Institute of Virology
Dr. Ambedkar Road,
Pune India – 411 001

Dr. Neena Valecha
Statutory Member
Director
National Institute of Malaria Research
Sector-8, Dwarka,
New Delhi – 110 077

Dr. Neeru Singh
Statutory Member
Director
Regional Medical Research Centre for Tribals
Nagpur Road, PO Garha,
Jabalpur – 482 003 (M.P)

Dr. Pradeep Das
Statutory Member
Director
Rajendra Memorial Research Institute
of Medical Sciences
Agamkuan, Patna – 800 007

Dr. G.S. Toteja
Statutory Member
Director
Desert Medicine Research Centre
New Pali Road, Jodhpur – 342 005

Dr. B.K. Tyagi
Statutory Member
Director-in-Charge
Centre for Research in Medical Entomology
No.4, Sarojini Street, Chinna Chokkikulam,
Madurai – 625 002

Dr. P. Vijayachari
Statutory Member
Director
Regional Medical Research Centre
Post Bag No.15, Port Blair – 744 101

Institutional Human Ethics Committee (IHEC) Members

Dr. C. Adithan
Chairman
Director-Professor & Head
Department of Pharmacology, JIPMER
Puducherry – 605 006

Dr. V. Balu
Member
Dean (Retd.)
Mother Theresa Institute of Health Sciences
Pudupet, Puducherry – 605 008

Dr. S. Gunasekaran
Member
Dean (Retd) of Humanities Studies
Pondicherry University
No.3, West Club Road,
Apartment B, Shenoy Nagar,
Chennai – 600 030

Dr. V. Govindaraj
Member
Medical Superintendent
Indira Gandhi Govt. General Hospital &
Post Graduate Institute
Puducherry – 605 001

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

Dr. M. Kalyanasundaram
Member
Scientist G
Vector Control Research Centre
Puducherry – 605 006

Dr. Shanthi Ananthakrishnan
Member
2–A Vairam Enclave,
Iyyanar Koil Street, Ellapillaichavadi,
Puducherry – 600 005

Dr. L.K. Das
Member Secretary
Scientist E
Vector Control Research Centre
Puducherry – 605 006

Institutional Animal Ethics Committee (IAEC) (w.e.f. May 2013)

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

Prof. V.N. Rao
Member
HOD of Veterinary Clinical Medicine
RGCV & AS, Puducherry

Dr. A. Yogamoorthi
CPCSEA Main Nominee
Reader, Dept. of Ecology & Environmental Sciences
Pondicherry University,
Puducherry

Prof. S.C. Parija
CPCSEA Link Nominee
HOD of Microbiology, JIPMER
Puducherry

Dr. B. Kumaran
Scientist from outside the Institute
Associate Professor in Zoology
Kanchi Mamunivar College for PG Studies, Lawspet,
Puducherry

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
Vector Control Research Centre
Puducherry – 605 006

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

Dr. K.P. Paily
Scientist In-charge of Animals facility
Scientist E
Vector Control Research Centre
Puducherry – 605 006

Equipment Purchase Committee

Dr. P.P. Mathur
Chairman
Professor & Head
Dept. of Biochemistry & Molecular Biology
Pondicherry University, Puducherry – 605 014

Dr. G. Ahila
Member
Professor & Head
Dept. of Computer Science
Pondicherry University, Puducherry – 605 014

Dr. K. Gunasekaran
Member
Scientist F
Vector Control Research Centre
Puducherry – 605 006

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

Mr. R. Joseph Suresh
Member Secretary
Administrative Officer
Vector Control Research Centre
Puducherry – 605 006

Building Committee

Dr. S. Sabesan
Chairman
Scientist G
Vector Control Research Centre
Puducherry – 605 006

Er. K. Kalyanasundaram
Expert Member
Retd Superintending Engineer (PWD)
No.44, Subramaniar Koil street, Kathirkamam,
Puducherry – 605 013

Er. N. Ayyadurai
Expert Member
Retd Superintending Engineer (PWD)
22, West Main Road, Brindavan Colony,
Puducherry – 605 013

Dr. K. Krishnamoorthy
Member
Scientist F
Vector Control Research Centre
Puducherry – 605 006

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

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

Accounts Officer
Member
Vector Control Research Centre
Puducherry – 605 006

Administrative Officer
Member
Vector Control Research Centre
Puducherry – 605 006

Section Officer (Maintenance)
Member Secretary
Vector Control Research Centre
Puducherry – 605 006

6.2 INTRA-INSTITUTIONAL COMMITTEES

Environmental Safety Committee / Biosafety Committee

Dr. L.K. Das	*Chairman*
Dr. A.M Manonmani	*Member*
Dr. K.P. Paily	*Member*
Dr. C. Sadanandane	*Member*
Section Officer (Maintenance)	*Member Secretary*

Library Committee

Dr. A.M. Manonmani	*Chairman*
Dr. A.R. Rajavel	*Member*
Dr. G. Rajendran	*Member*
Dr. Nisha Mathew	*Member*
Dr. R. Srinivasan	*Member*
Mrs. R.Sundarammal	*Member Secretary*

Purchase Committee

Dr. K. Gunasekaran	*Chairman*
Dr. K.P. Paily	*Member*
Dr. C. Sadanandane	*Member*
Dr. V. Vasuki	*Member*
Dr. L.K. Das	*Member*
Section Officer (Stores)	*Member Secretary*

Equipment Maintenance Committee

Dr. K.P. Paily	*Chairman*
Dr. Nisha Mathew	*Member*
Dr. S. Subramanian	*Member*
Dr. K.H.K. Raju	*Member*
Mr. G. Prabhakaran	*Member*

Condemnation/Auction Committee

Dr. K. Krishnamoorthy	*Chairman*
Dr. A.M. Manonmani	*Member*
Dr. S. Subramanian	*Member*
Dr. A. R. Rajavel	*Member*
Accounts Officer	*Member*
Section Officer (Stores)	*Member Secretary*

Vehicle Maintenance Committee

Dr. S. Subramanian	*Chairman*
Dr. A.R. Rajavel	*Member*
Dr. C. Sadanandane	*Member*
Dr. G. Rajendran	*Member*
Mr. T. Mariappan	*Member Secretary*

Grievance/Staff Welfare Committee

Dr. M. Kalyanasundaram	*Chairman*
Dr. P. Vanamail	*Member*
Dr. I. Geetha	*Member*
Mr. T. Mohanan	*Member*
Mr. K. Karunakaran	*Member*
Mr. S. Balasubramanian	*Member Secretary*

Committee for Prevention of Sexual Harassment of Women in Work Place

Dr. V. Vasuki	*Chairman*
Dr. R. Srinivasan	*Member*
Dr. B. Nandha	*Member*
Mrs. K. Vijayalakshmi	*Member*

Official Language Implementation Committee

Dr. L.K. Das	*Chairman*
Dr. B. Nandha	*Member*
Mr. K.H.K. Raju	*Member*
Mr. B. Kumaresan	*Member*
Mr. Y. Srinivas Murty	*Member Secretary*

Residential Quarters Allotment Committee

Dr. K. Krishnamoorthy	*Chairman*
Dr. K.P. Paily	*Member*
Dr. V. Vasuki	*Member*
Dr. C. Sadanandane	*Member*

General Maintenance Committee

Dr. R.L.J. De Britto	*Chairman*
Dr. R. Srinivasan	*Member*
Dr. S. Poopathi	*Member*
Dr. V. Vasuki	*Member*
Mr. K. Sundararajan	*Member Secretary*

Management Committee

Dr. M. Kalyanasundaram	*Chairman*
Dr. S. Sabesan	*Member*
Dr. K. Krishnamoorthy	*Member*
Dr. K. Gunasekaran	*Member*
Dr. L.K. Das	*Member*
Dr. A.M. Manonmani	*Member*
Administrative Officer	*Member*
Accounts Officer	*Member*
Mrs. B. Parassacty	*Member Secretary*

7 Staff Position

DIRECTOR
Dr. P. Jambulingam

Scientific

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

Library

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

Administration & Accounts

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

Technical

Mr. A. Elango	*Technical Officer - A*
Mr. G. Jeeva	*Technical Officer - A*
Mr. V. Padmanabhan	*Technical Officer - A*
Dr. (Mrs.) K. Athisaya Mary	*Technical Officer - A*
Dr. (Mrs.) B. Nandha	*Technical Officer - A*
Dr. (Mrs.) Ambilikumar	*Technical Officer - A*
Mrs. K.S. Snehalatha	*Technical Officer - A*
Mr. R. Natarajan	*Technical Officer - A (Adhoc)*
Dr. (Mrs.) A. Krishnakumari	*Technical Assistant (Research)*
Mrs. Abidha	*Technical Assistant (Research)*
Mr. T. Vijayakumar	*Technical Assistant (Research)*
Mr. G. Prabakaran	*Technical Assistant (Research)*
Dr. K.N. Vijayakumar	*Technical Assistant (Research)*
Dr. (Mrs) I. Geetha	*Technical Assistant (Research)*
Dr. N. Sivagnaname	*Technical Assistant (Research)*
Mr. M. Palaniyandi	*Technical Assistant (Research)*
Dr. K. Harikishan Raju	*Technical Assistant (Research)*
Mrs. T. Sankari	*Technical Assistant (Research)*
Mr. S. Muthukumaravel	*Technical Assistant (Research)*
Mr. N. Krishnamoorthy	*Technical Assistant (Research)*
Mr. A. Mathivanan	*Technical Assistant (Research)*
Mr. K. Vaidyanathan	*Technical Assistant*
Mr. S. Kandasamy	*Technical Assistant*
Mr. K. Vivekanandan	*Technical Assistant*
Mr. V. Ramu ***	*Technical Assistant*
Mr. K. Mathivanan	*Technical Assistant*
Mrs. Regnakumari Packirisamy	*Technical Assistant*
Mr. B. Edwin	*Technical Assistant*
Mr. G. Sritharan	*Technical Assistant*
Mr. Md. Mustafa Baig	*Technical Assistant*
Mrs. T. Sonia	*Technical Assistant*
Mrs. G. Vijayalakshmi	*Staff Nurse*

** transferred to RMRC, Belgaum*
*** retired from service on VRS*
**** Retired from service on superannuation*