

HIGHLIGHTS

JAPANESE ENCEPHALITIS

Japanese encephalitis (JE) is the most important cause of viral encephalitis in eastern and southern Asia with 30,000–50,000 cases reported annually and JE epidemics occur in entire South East Asia regularly every year. However, at present, there is little national capability of facing JE outbreaks and most of the measures that are undertaken during the epidemics are of little consequence in view of high morbidity and mortality. There is, therefore, a requirement of a reliable “Early Warning System” to forecast JE outbreaks. Centre for Research in Medical Entomology and Indian Institute of Chemical Technology (IICT), Hyderabad collaborated on a research project to develop a forecasting model for impending JE epidemics. This was accomplished through necessary entomological and serological studies in Kurnool district, which is one of the highly JE-endemic districts in Andhra Pradesh. A total of 2235 female mosquitoes were collected in dusk collections in urban areas. *Culex tritaeniorhynchus* was the dominant species comprising 74.9% of the total sample collected, followed by *Cx. gelidus* (20.1%), *Cx. quinquefasciatus* (2.75%) and *An. subpictus* (1.27%). In rural areas, a total of 6786 mosquitoes belonging to 4 genera and 9 species were collected in dusk collections: *Cx. tritaeniorhynchus* (84.8%), *Cx. quinquefasciatus* (7.5%) and *An. subpictus* (5.38%). *Culex gelidus*, which was common in urban areas, constituted only 0.6% of the total catch in rural areas. Abundance (females /man-hour) of the major JE vector *Cx. tritaeniorhynchus* was lowest during summer months (May and July) and peaked in (November). The PMH density of *Cx. gelidus* peaked in the month of September in urban area and November in rural areas. However, *Cx. gelidus* abundance was found to be very low in rural areas when compared to that in the urban area. Natural JE virus infection was found in *Cx. tritaeniorhynchus* and *Cx. gelidus*. Two pools of *Cx. tritaeniorhynchus* in September 2005, six pools in November 2005 were found positive for JE virus. *Culex gelidus* collected, one pool each in September and November 2005, were found positive for JE virus, indicating active transmission in the study area.

Vector abundance and JE virus activity was monitored longitudinally from the endemic villages of Cuddalore district, Tamil Nadu. Two peaks of abundance of *Cx. vishnui* subgroup mosquitoes were observed; the October 2005 peak being very prominent with 653.75 PMH and a comparatively less prominent in January 2006 (447.50 PMH). Overall MIR decreased in this year from 0.714 to 0.386. JEV infection was found in the vectors on all the alternative month from May 2005 onwards. High infection rate (MIR) was recorded during the month of May (6.667) and August (2.105).

In the case of molecular biological studies, total protein of *Toxorhynchites splendens* was resolved in a 10%SDS-PAGE. Blotting experiment was carried out to transfer the protein from the gel to the PVDF membrane. Virus overlay protein binding assay (VOPBA) was done with JEV antigens and JEV monoclonal antibodies. The study has shown that the JEV antigen was found to bind to a putative 32 kD protein of *Tx. splendens*. In order to examine the necessity of the receptor for virus binding, attempts are being made to block the receptor protein of JEV with the rabbit immune serum.

LYMPHATIC FILARIASIS

During course of evaluation of the role of vector control (VC) and mass drug administration (MDA) in the filariasis elimination campaign, microfilaraemia prevalence (MFP) was found to be 0.58% and 3.02% in 2-5 and 15-25 years, respectively, in MDA alone villages, while in MDA+VC villages nil MFP was noted in 2-5 years. The AGP was 3 times lower in the MDA+VC arm as compared to MDA alone arm in both the age groups. In children the MFP maintained at zero level, while there was 71% reduction in AGP after 5 MDAs. The vector control villages continued to demonstrate negligible vector density with nil transmission, both in the resting and landing population. In these villages, the average PMH resting density during April 2005-March 2006 was 0.15, while in villages without vector control it was 24.06, and the transmission intensity index (TII) was 0.1008.

In the WHO/TDR funded study at Tirukoilur, no MDA was carried out during the year 2005. However, after 3 rounds of MDA longitudinal monitoring in the study villages had indicated greater reductions in the prevalence and intensity of microfilaria in DEC+ALB arm compared to DEC alone arm. After one year of MDA IV, the cumulative percentage reduction was similar (60%) in both the arms. But amongst the 3 age groups, 6-9 years showed higher MFP reduction in DEC+ALB arm alone. Decline in antigenaemia levels was in favour of DEC+ALB arm, where the adult age group 10-25 years demonstrated 2 times higher reduction than in the DEC alone arm. In a cohort of microfilaraemic survey, 85% and 79% subject were cleared of microfilaria in DEC+ALB and DEC alone arms, respectively. Nil antigenaemia was recorded in DEC+ALB arm in those children born after initiation of MDAs (in 2 & 3 years), while not in DEC alone arm.

In landing collection, the average number of infective mosquitoes biting per month dropped from 4.6 to 1.9 after 4 MDAs in DEC+ALB, whereas from 9.0 to 2.6 in DEC alone arm. In both arms transmission indices (TII & TP) were found to be low after MDA IV and the overall reductions of 90% and 88% were recorded in DEC+ALB and DEC alone arms respectively.

The prevalence and intensity of soil transmitted helminth infections, viz, *Ascaris*, hookworm and *Trichuris*, was determined by Kato Katz method in school children aged 9 and 10 years old. After one-year post MDA IV, the prevalence due to any of the 3 worms was reduced by 78% in DEC+ALB arm ($P < 0.0001$), while 3 times less in DEC alone arm. In the latter arm, the reduction was due to the impact of DEC on *Ascaris*. The egg intensity declined as 98% and 68%, respectively in DEC+ALB and DEC alone arms ($P < 0.0001$). For individual helminths, a reduction of 79%, 87% and 67%, respectively, was recorded for *Ascaris*, Hookworm and *Trichuris* in DEC+ALB arm. DEC alone did not demonstrate any impact on hookworm and *Trichuris* infections.

DENGUE

Dengue fever (DF) has in recent years emerged as a major mosquito-borne infection of great public health concern in Southeast Asia, in particular. However, for past two decades there has been a sharp increase in frequency of DF epidemics, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). A three year WHO/TDR-funded research project on dengue entitled "Community based dengue vector control under health system in one district (south India) and transfer of technology" was started in 2002. "Evaluation phase" (Feb 2005-Aug 2005) of the project was carried out to assess the impact of community-based dengue vector control programme by using IEC, sociological and entomological evaluation. Based on the KAP surveys conducted, it was clearly found that the community awareness had increased significantly in intervention (INT) areas on DF, including transmission by the vector mosquito, *Aedes aegypti*, and its breeding habitats. Difference in larval indices was observed between check (CHK) and intervention areas. There was a general reduction in larval indices in intervention areas when compared to check areas. In the CHK area, house index (HI) was higher in all months when compared to intervention areas and the mean HI was 14.42, whereas in intervention areas HI was 10.88. Similarly, Breteau Index was higher in CHK area (range 912.75-27.0; mean 13.88) compared to that in intervention areas (range 11.0-18.5; mean 19.46). *Aedes aegypti* breeding in different types of habitats was compared in INT and CHK areas. The percentage positivity of *Ae. aegypti* breeding was found higher in check areas than in intervention areas. The pupal index shows that there was considerable difference between INT areas (range 3.0-17.75) and CHK area (22.5-147.5). It clearly shows that the IEC-based intervention strategies can make appreciable impact on community knowledge on dengue fever, and resultantly on its prevention.

Random amplified polymorphic DNA (RAPD) technique was used to determine the genetic diversity of the *Aedes aegypti* mosquitoes prevalent in Tamil Nadu. Standardization of DNA extraction from various mosquito populations and the RAPD PCR were carried out. The *Aedes aegypti* RAPD profile was studied with the set of 20 commercially available primers. Of those three primers were selected for analyzing the freshly emerged adults. Immatures were collected from different parts of Tamil Nadu and Kerala (Tiruppur, Trichy, Virudachalam, Madurai, Rameshwaram and Trivandrum). The RAPD results have shown that the close proximity between populations collected from distant places.

VECTOR CONTROL

Some species of bacteria, of the genus *Pseudomonas*, including *P. fluorescens* are used for the control of soil-borne or foliar pathogens. A mosquitocidal flowable formulation developed from the metabolite(s) of *P. fluorescens* was tested against five species of vector mosquitoes viz., *Anopheles stephensi* (malaria vector), *Aedes aegypti* and *Ae. albopictus* (dengue vectors), *Culex quinquefasciatus* (filariasis vector) and *Cx. tritaeniorhynchus* (Japanese encephalitis vector). The laboratory bioassay studies show that all the vector species tested are susceptible to the metabolite(s) of *P. fluorescens*. The larvicidal and the unique pupicidal efficacy of the formulation indicated excellent mosquito control potential of the formulation.

OUTBREAK INVESTIGATIONS

During 2005 Gorakhpur faced a devastating JE epidemic outbreak affecting 6097 individuals with 1398 deaths. In intensity and magnitude, this epidemic surpassed all previous reported epidemics. A team from CRME visited Gorakhpur in November, 2005 to carry out epidemiological investigation during the course of the epidemic. Entomological surveys were conducted in the affected villages which include adult (dusk collection) and larval (in and around paddy ecosystem) collections. A total of 14 different species of mosquitoes namely *Culex tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. fuscocephala*, *Cx. gelidus*, *Cx. quinquefasciatus*, *Ma. annulifera*, *Ma. indiana*, *Ma. uniformis*, *An. barbirostris*, *An. hyrcanus (gr)* and *An. peditaeniatus* were collected. Species of *Culex vishnui* subgroup and *Cx. gelidus*, the primary vectors of Japanese encephalitis, consisted 13% of the total mosquitoes collected. Wild-caught female mosquitoes were tested for JE virus by using antigen capture ELISA and Insect bio-assay. All the mosquito samples were found negative for JE virus.

Intravenous blood samples were collected from the patients clinically diagnosed as encephalitis and admitted in the district hospital, Gorakhpur. The age distribution of patients ranged from 8 months to 10 years and majority of the patients (65.6%) were female children. The samples were tested for JEV-IgM antibodies by using MAC ELISA kits. All the 32 samples were found negative for JEV-IgM antibodies in both tests. The results were confirmed with MAC ELISA kits obtained from National Institute of Virology, Pune, India.