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Commentary

Detection of dengue viral infections in *Aedes* mosquitoes: An essential tool for epidemiological surveillance

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are important mosquito-borne viral diseases of humans and recognised as important emerging infectious diseases in the tropics and subtropics[1]. There has been a considerable increase in the geographic spread, number of cases and severity of the disease in the past four decades, since there is a drastic change in the environment due to rapid urbanization and increase in transportation facilities in several parts of India. Thus, the disease once confined to urban areas has now penetrated in to rural areas with several outbreaks from rural areas.

As there is no vaccine available for the dengue fever, the prevention and control of the disease mainly depends on the epidemiological surveillance that provides reliable estimate of the disease and thereby helping to implement effective vector control measures. There are several methods such as detection of virus specific IgM antibodies, virus isolation and viral antigen detection available for the diagnosis of the clinical specimens. However, it is important to find out the vector infection rate as well as the density in an area to determine the transmission potential of dengue virus. Identifying serotypes only during outbreaks will not be of much helpful to prevent any future outbreaks. Mapping of areas with prevalence of different serotypes is essential to develop a forecasting system. In India, dengue virus isolations in mosquitoes have been made in wild-caught mosquitoes as early as 1960s[2,3]. However, suckling mice inoculation for virus isolation, a laborious and time consuming technique which requires many passages has been replaced by recent techniques such as immunofluorescent assay using serotype specific monoclonal antibodies.

Immunocytochemical studies on the distribution of dengue-3 (DEN-3) virus in female *Aedes aegypti* mosquitoes showed foregut, salivary glands and nervous tissue as the major tissues infected[4]. Natural vertical transmission of dengue virus in *Ae. aegypti* plays an important role in maintaining the endemicity of virus even during inter-epidemic period[5]. However, the epidemiological significance of transovarial transmission is not known, as there is no direct evidence to prove that a mosquito infected through transovarial transmission causes apparent dengue infections.

Indirect immunofluorescent assay (IIFA) on individual specimens of *Ae. aegypti* has also provided valuable information on vector infection with dengue virus during outbreak period[6,7]. Although it is more labour intensive than detection of dengue viral infections in pooled mosquitoes, it provides precise estimate of vector infection rate for a particular geographic area[8]. However, in dengue surveillance programme this method will not be much useful as we need to screen a large volume of specimens collected from different geographical areas in different seasons routinely.

In this issue, Angel & Joshi[9] have reported the prevalence of different serotypes of dengue viruses in four endemic districts of Rajasthan, India. Totally, 498 adults of *Ae. aegypti* collected in different geographical locations have been tested individually for the presence of dengue virus by IFA. But this needs to be extended to other areas also and the mosquito collections should be carried out longitudinally to find out the seasonal differences in vector densities as it plays an important role in the transmission risk of the disease by establishing man-mosquito contact. It is important to find out the combinations of serotypes prevalent in an area within a specified period. For forecasting any DHF outbreak, the prevalence of different serotypes should be continuously monitored by virological surveillance.

For continuous monitoring of dengue virus in vector mosquitoes, collaboration with the State Public Health System would be required. Japanese encephalitis (JE) surveillance network has been well established in Tamil Nadu by the Department of Public Health & Preventive Medicine, Chennai, with Centre for Research in Medical Entomology (CRME), Madurai, for the past 10 years where the dried specimens of JE vectors collected in the field by the Zonal Entomological Teams are sent to the CRME for detection of JE viral antigen by ELISA[10]. Similarly, for monitoring dengue virus infections using desiccated specimens of *Ae. aegypti,* dengue-antigen capture EIA on specimens stored at room temperature (31-34°C), a field-friendly procedure for storing and transporting specimens, has been standardized at the CRME[11] and the collaboration with the Department of Public Health & Preventive Medicine, Chennai, has been recently made for receiving and analysing the specimens in dengue surveillance programme in Tamil Nadu (Personal communication with Dr V. Thenmozhi, CRME). It is thus understood that the laboratory facilities available at the central research laboratories at different States have to be utilised in JE and dengue surveillance system which will provide a comprehensive technical guidance and support to the State and district health functionaries.

Several laboratory methods such as suckling mice inoculation, antigen capture ELISA, IIFA on individual mosquitoes, Toxo-IFA, cell culture techniques and polymerase chain reaction have been well used on several occasions to detect the dengue viral infections in wild caught mosquitoes. However, it is imperative to identify the method which could be used in the epidemiological surveillance. Angel & Joshi[9] have detected dengue virus in individual mosquito head squashes and the remnants of positive mosquitoes have been used in cell culture and mice inoculation followed by IFA. It is not clearly understood whether there was a complete correlation of results among various methods used.

Sithiprasasna *et al*[8] detected dengue viruses using indirect ELISA in *Ae.aegypti* and *Ae.albopictus* adults. They suggested that ELISA was faster and less labour intensive than mosquito inoculation and this method could be used for screening large number of vectors. Further, the detection of dengue viral antigen by ELISA depends on the accumulation of detectable levels of antigen. Mosquito pool size and interpretation of results based on OD values should be standardized in each laboratory. Some authors have considered the pool positive if the OD $\geq$ mean + 4SD of laboratory reared uninfected mosquitoes[12] and others have used OD $\geq$ mean + 3SD of uninfected mosquitoes[8,13]. Besides *Ae. aegypti*, *Ae. albopictus* has also been found positive for dengue viral infections on several occasions[12,14,15]. Hence, it is emphasized to screen *Ae. albopictus* adults also when sizeable numbers are collected in the field. The advantages and disadvantages of various methods available for detection of dengue virus infections in mosquitoes have been reviewed and it has been suggested that ELISA can be used in dengue surveillance[16].

The main objectives of detecting dengue viral infection in wild-caught mosquitoes are, *(i)* to find out the prevalence of different serotypes that are circulating in the community, and *(ii)* to develop a forecasting system for DF/DHF outbreaks in an area. Considering the laboratory burden and time, IFA on individual specimens as done by Angel & Joshi[9] would not be much useful in routine surveillance programme. ELISA could be an ideal method for rapid screening of large number of pooled specimens, and the positive pools may be subjected for Toxo-IFA system[17] or cell culture methods to identify the serotype of dengue virus. An antigen capture ELISA recently developed for detecting dengue viral antigen in desiccated mosquitoes[11] would be much useful in dengue surveillance. This could be considered as an essential component to establish a Dengue Surveillance Network for collection, compilation and documentation of data to develop a model for forecasting DF/DHF outbreaks.

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