Serological & entomological investigations of an outbreak of dengue fever in certain rural areas of Kanyakumari district, Tamil Nadu

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Background & objectives: During the first week of July 2003, suspected cases of dengue fever were reported from three villages in Kanyakumari district in Tamil Nadu. Since the fever outbreak occurred for the first time in these villages, serological, virological and entomological investigations were carried out to confirm the aetiology of outbreak.

Methods: A total of 76 plasma samples were collected from suspected cases of dengue fever and screened for the presence of IgM antibodies by Pan Bio ELISA kit. Toxo-IFA system was used for the isolation of dengue virus from the plasma samples. Vector survey employing ovitraps and adult landing collection were carried out in the study villages. Pooled samples of *Aedes* mosquito were screened for dengue virus antigen by an in-house antigen capture ELISA test employing dengue virus specific monoclonal antibodies.

Results: Of the 76 samples tested, 15 (20%) were found positive for dengue virus specific IgM antibodies. Dengue virus serotype-3 was detected from a plasma sample by Toxo-IFA test using virus specific monoclonal antibodies. Entomological survey revealed the abundance of *Aedes albopictus* (Skuse) mosquitoes in the study area. One pool consisting of 12 *Ae. albopictus* males were found positive for dengue virus infection.

Interpretation & conclusion: Based on the IgM antibody capture ELISA results, it was evident that the current infection was caused by dengue virus in the affected areas. All the age groups were affected during this outbreak. Detection of dengue virus serotype-3 in plasma samples further confirmed the aetiology of this outbreak. The high prevalence of the mosquito vector *Ae. albopictus* (Skuse) was observed. Detection of dengue virus antigen in the male mosquitoes confirms that the virus is maintained in wild populations of *Ae. albopictus* in these areas.

Key words Dengue - outbreak investigation - seroepidemiology - vector - viral antigen detection
Dengue, a mosquito-borne flavivirus infection of humans is caused by four serologically distinct viruses namely dengue virus -1, 2, 3 and 4. Dengue and its severe manifestations - dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)\(^1\), are the serious public health problems in the tropics\(^2\). In India, dengue virus infections has been frequently encountered in epidemic proportions in several States\(^3\)-\(^7\). In southern India, the disease has been reported in Tamil Nadu\(^7\), Karnataka\(^8\), Andhra Pradesh\(^9\) and Kerala\(^10\). In Tamil Nadu, circulation of all the four serotypes of the dengue viruses have already been documented\(^11\). Recently, dengue infection has been recognized in newer areas of Tamil Nadu from where cases have never been reported earlier. Moreover, the increasing trend of dengue virus activity in southern India and its warning signal of developing future epidemics has been reported\(^12\). During July 2003, suspected cases of dengue fever were reported from certain rural areas of Kanyakumari district, Tamil Nadu. Hence, serological and virological investigations were done to confirm the aetiology and entomological survey to find out the prevalence of vector *Aedes* mosquito in these areas.

**Material & Methods**

**Study area:** During the first week of July 2003, cases of suspected dengue fever were reported in Moopuvilai (with a population of 893) village of Melpuram primary health center (PHC), and Venganamkode (616) and Pacode (630) villages of Edaicode PHC under Melpuram block of Nagercoil health unit district (HUD) (8.11 N and 77.29 E), Kanyakumari district of Tamil Nadu. The affected area is geographically located in the sylvan environment of the Western Ghat region at an altitude of about 100 meters with extensive rubber plantations. Relative humidity and temperature varied between 70-90 per cent and 22ºC to 35ºC, respectively. The area receives rainfall during both southwest (May - July) and northeast (September - November) monsoons, with an annual rainfall of up to 350 cm. Though the main occupation of the people in the area is to cultivate rubber plantations, some move to work in the neighboring Kerala State and visit their houses in weekends.

**Serological investigations:** A few suspected cases with fever, headache, arthralgia, myalgia were initially screened at the Institute of Vector Control and Zoonosis (IVC&Z), Hosur, Tamil Nadu using an in house IgM antibody capture (MAC)-ELISA. In the present study, 76 peripheral blood plasma samples (0.5 ml) were collected by finger prick method. Of the 76 plasma samples collected, 50 were from fever cases with clinical symptoms suggestive of dengue infection attending Thakkalai government hospital, where a “Dengue ward” was functioning during the outbreak, and the remaining 26 samples were collected from the surrounding areas of three villages. These samples were transported to the Centre for Research in Medical Entomology (CRME), Madurai in wet ice and were screened against dengue virus specific IgM antibodies by Pan Bio MAC-ELISA Kit (Brisbane, Australia) as per the manufacturer’s instructions. The acute plasma samples were inoculated in *Toxorhynchitis splendens* larvae and incubated at 37ºC for 14 days. Dengue virus antigen was screened in the head squashes of the *Toxorhynchites* larvae by immunofluorescence antibody (IFA) test\(^13\) employing dengue virus serotype specific monoclonal antibodies\(^13\) [DEN-1=D2-IFI-3, DEN-2=3H5-1-21, DEN-3=D6-8A1-12, DEN-4=IH10-6-7 kindly gifted by D.J.Gubler, Centers for Disease Control and Prevention (CDC), Atlanta, USA].

**Entomological investigations:** Entomological investigations were carried out to understand the role of mosquito vectors in dengue virus transmission by monitoring the vector density, man-mosquito contact and viral antigen circulation among wild vector population. Ovitrap method (considered as the most sensitive and effective tool for *Aedes* surveillance) was used to study the prevalence of dengue vectors\(^14\)-\(^15\). This was carried out by placing 25 ovitraps in Moopuvilai, 10 in Venganamkode and 15 in Pacode village. In each locality, the traps were placed in the peridomestic area within a radius of about 500 metres around the house from where dengue cases were reported.

Ovitraps were exposed over 24 h commencing from 0700 h. Adult collection was carried out in outdoor while landing on human to know the man-
mosquito contact. This was carried out in the forenoon between 0900 and 1100 h by spending 4 man hours at Venganamkode and in the afternoon between 1530 and 1630 h at Moopuvilai by spending 2 man hours. The prevalence (i.e., proportion of traps with eggs) and intensity (i.e., number of eggs per positive traps) of egg population and the mean egg density (prevalence X intensity) were calculated. The traps were transported to CRME, Madurai, after an interval of three days. After counting, the eggs were reared and identified after emergence. Pooled samples of adult Aedes mosquitoes (both wild caught and those reared from larvae in laboratory) were screened for the presence of dengue virus antigen by an in-house antigen capture ELISA employing monoclonal antibodies13.

Results & Discussion

The MAC-ELISA test detected the dengue virus specific IgM antibodies from 15 (9 males, 6 females) cases (Table I) indicating the dengue virus infection. Most of the patients were in the age group of 6-15 yr (24.14%). It was observed that more females (41 cases) were affected than males (35). Similarly in Dharmapuri district of Tamil Nadu, more females were affected than males17.

Of the six plasma samples inoculated in the Toxorhynchites splendens larvae, one was found positive for dengue virus type-3 by IFA test employing the serotype specific monoclonal antibodies. During the study period, dengue serotype-3 was also isolated from plasma samples collected in Kerala, which is border area of Nagercoil, Tamil Nadu State10.

Altogether 3,498 mosquito specimens consisting of six species were collected. Among these Ae. albopictus was the predominant species representing 99 per cent of the total catch (Table II). A total of 3,379 eggs of Ae. albopictus were collected through 50 ovitraps in the three villages. The egg density was very high in all the three localities as the mean intensity recorded was over 52.2 (Table III). Ae. aegypti, the primary vector of dengue was not seen during this survey. A total of 36 pools consisting of 1,725 adult specimens (62 wild caught adults and 1,663 reared adults) of Ae. albopictus were screened by antigen capture ELISA for dengue virus infection. Of these, one pool consisting of 12 males (5 wild caught and 7 reared adults) was found positive for

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<th>Table II. Surveillance of the mosquitoes in the study area</th>
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<tr>
<td>Species</td>
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<tr>
<td>Aedes albopictus</td>
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<tr>
<td>Ae. vittatus</td>
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<td>Ae. jamesi</td>
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<tr>
<td>Armigeres subalbatus</td>
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<tr>
<td>Culex quinquefasciatus</td>
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<td>Toxorhynchites splendens</td>
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dengue virus antigens. Detection of dengue virus antigen in the male mosquitoes could probably explain the maintenance of dengue virus in nature through vertical transmission, which has epidemiological significance. Thenmozhi et al\(^{13}\) documented the occurrence of vertical transmission of dengue virus in male Ae. aegypti mosquitoes at Vellore, Tamil Nadu. The results of ovitraps studies showed the predominance of Ae. albopictus in the three areas surveyed. The relative abundance of Ae. albopictus was found to be higher (79.1) at Moopuvilai, when compared to other areas [Venganamkode (52.2) and Pacode (58.6)] (Table III). The breeding of vector was seen to be supported by the rubber collection cups where rainwater stagnates. Mosquito larvae were also collected from the axils and other parts of the plants collecting water.

After critical verification of the available data, it was evident that the suspected dengue cases were first reported in Kerala during June 2003. Later the cases appeared in Nagercoil (during July 2003). Moreover, the frequent movement of migratory workers from Kerala to Nagercoil (approximate distance 50 km) has also contributed in this outbreak. Some of the migratory workers who visited Kerala for job were reportedly returned to study areas in the weekend with symptoms of suspected dengue fever. Unfortunately clinical samples from these workers were not available for analysis. Other serologically confirmed dengue cases were reported around the migratory workers residence. From this evidence, we presumed that the dengue virus could have been introduced from Kerala to Nagercoil through man or mosquito. However, this needs further confirmation. Cross-border dissemination of dengue virus from Maharashtra to the neighbouring villages in Gujarat and Madhya Pradesh has been observed\(^{19}\). Victor et al\(^{17}\) reported the possible introduction of dengue virus by the labourers from Coimbatore to the Mampatti village of Dharmapuri district in Tamil Nadu. Similarly the transmission of various infectious diseases due to cross-border movement of large populations have been reported\(^{20}\). Further, systematic surveillance of dengue infection and inclusion of molecular methods in virological surveillance would probably answer many questions in this area.

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References


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Aedes albopictus