Natural vertical transmission of dengue viruses by *Aedes aegypti* in Chennai, Tamil Nadu, India


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**Background & objectives:** Dengue viruses are spread and maintained in an *Aedes aegypti*-human-*Ae. aegypti* cycle in urban areas of the tropics. Dengue viruses are also maintained in nature by vertical transmission by *Ae. aegypti*. A study was undertaken in Chennai, a known endemic city in south India, to comprehend the natural vertical transmission dynamics in *Ae. aegypti* and to assess its epidemiological importance.

**Methods:** *Ae. aegypti* males collected in resting and landing collections were tested for dengue virus infection by antigen-capture enzyme-linked immunosorbent assay (ELISA) and further examined by insect bioassay, *Toxorhynchites splendens* inoculation-indirect immunofluorescence technique (Toxo-IFA) using serotype-specific monoclonal antibodies (Mabs), if found positive by ELISA.

**Results:** Of the 509 pools of *Ae. aegypti* males (n=5408) screened, 15 pools, collected in April, June-July, November-December in 2003 and March, May in 2004, were found positive for dengue virus infection and the minimum infection rate (MIR) among adult males was high in June 2003 (28.0/1000). Three positive pools could be serotyped as dengue-2 (2 pools) and dengue-3 (1 pool).

**Interpretation & conclusions:** Dengue virus isolations from wild caught males of *Ae. aegypti* indicate the occurrence of transovarial transmission. Vertical transmission was mainly observed in summer months when dengue infections in humans were low suggesting that dengue viruses adopt a novel strategy of surviving adverse climatic conditions.

**Key words** *Aedes aegypti* - dengue virus - ELISA - vertical transmission

Dengue is one of the most important emerging tropical diseases. Dengue epidemics have been reported in over 100 countries and 2.5 billion people live in areas where dengue is endemic. The dengue viruses are spread and maintained by *Aedes aegypti*, the principal vector of dengue. *Ae. aegypti* is an anthropophilic mosquito, which has evolved intimate relationship with humans and exhibits several behavioural traits like oviposition in man-made and man-used natural and artificial containers. Although, the vector-man-vector transmission is well understood mechanism of transmission of dengue viruses under natural...
conditions, there are reports of vertical transmission taking place in certain circumstances particularly during adverse climatic conditions. In India, the reports of natural vertical transmission of dengue viruses are scarce, although the field evidence supporting vertical transmission as a means of persistence has been obtained for dengue virus. Vertical transmission of dengue was observed only in female progeny in experimental studies and no infection was observed in wild caught male mosquitoes in Jalore, India and it was suggested that the virus could not multiply in male mosquitoes. Therefore, a study was undertaken in Chennai, a known endemic city in south India, to comprehend the natural vertical transmission dynamics in Ae. aegypti and to assess its epidemiological importance.

Chennai, a coastal, metropolitan city, is the study area, having an area of 216 km², with 5.9 million populations. Dengue cases are reported every year and there was a dengue outbreak in 2001. The city receives rains from June onwards, however, mainly from northeast monsoon (September to November) with an average annual rainfall of 1245.7 mm. Chennai city experiences the highest temperature (varies between 38°C to 42°C) in May and June, and the monsoon failures and shortage in the collection of water in reservoirs lead to irregular and interrupted potable water supply. Householders are compelled to store water in permanent/temporary containers to meet their hardship.

**Material & Methods**

This study was carried out in Chennai during March 2003 to December 2004. The ethics committee of the Centre for Research in Medical Entomology (Indian Council of Medical Research), Madurai, India, approved the study protocol. Mosquitoes were collected from daytime landing collection of *Aedes* mosquitoes on human bait (after obtaining informed consent from volunteers and our own staff), and from resting collection of mosquitoes inside the houses. *Ae. aegypti* males were tested, in single species pools of 1 - 20, for dengue virus infection by antigen-capture enzyme-linked immunosorbent assay (ELISA), using dengue virus specific monoclonal antibody (MAb) D3-5C9-1, and detector MAb 6B6C-1 conjugated with horse radish peroxidase; broadly reactive against flaviviruses. If any pool was found positive by ELISA, it was further examined by the *Toxorhynchites splendens* inoculation-indirect immunofluorescence technique (Toxo-IFA) using serotype-type specific MAbs D2-IFI-3 (Dengue-1), 3H5-1-21 (Dengue-2), D6-8A1-12 (Dengue-3) and IH10-6-7 (Dengue-4) (Gifted by Barbara W. Johnson, CDC, USA). All the chemicals used in the study were procured from Sigma, USA. Dengue virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females mosquitoes tested.

**Results & Discussion**

Of the 509 pools (n=5408) of male *Ae. aegypti* screened by ELISA and IFA, 15 pools, collected in April, June-July, November-December in 2003 and March, May in 2004, were found positive for dengue virus infection. Three positive pools could be serotyped as dengue-2 (2 pools) and dengue-3 (1 pool). Although, MIR ranged from 2.7 (November, 2003) to 28.0 (June, 2003), dengue virus infection among adult male mosquitoes was higher (28.0 and 13.4) during June and July, the hot and dry months of the year (Fig.).

Vertical transmission of dengue viruses by *Ae. aegypti* has been reported in different geographic regions of the world including India. Dengue-2 and dengue-3 virus isolations from wild caught male *Ae. aegypti* indicated the occurrence of vertical transmission of dengue viruses in *Ae. aegypti* in Chennai. Dengue-2 has also been isolated from adult *Ae. aegypti* collected as larvae from natural breeding sites in Myanmar. Dengue-4 virus was recovered from adult *Ae. aegypti* reared from eggs collected in nature in Trinidad. But in the present study, wild caught male mosquitoes were found infected with dengue viruses. Dengue-2 and dengue-3 viruses were recovered from wild caught adult *Ae. aegypti* males and adult *Ae. aegypti* collected as larvae from natural habitats and reared to adults in Vellore, India.

Vertical transmission, in which the virus is transmitted from an infected female mosquito to her eggs as they pass through the genital tract, is known

![Fig. Dengue virus infection in male *Aedes aegypti* in Chennai.](image-url)
to support the persistence of some arboviruses in nature as a possible mechanism of surviving long period of adverse climatic conditions in Aedes transmitted arboviruses. Thenmozhi et al recorded vertical transmission of dengue virus in Ae. aegypti during the period when there was no apparent dengue infection in the villages near Vellore, Tamil Nadu. The infection rate in males of Ae. aegypti was found to be high in the summer months (June and July) and it can be concluded that vertical transmission of dengue viruses by Ae. aegypti helps for the maintenance of dengue viruses in nature especially during inter epidemic period when the vector density was low. The present study also revealed the circulation of more than one dengue virus serotypes in Chennai, which ascertains that this area is hyperendemic for dengue.

It is noteworthy that though the seasonality of the traditional Ae. aegypti-human-Ae. aegypti transmission of dengue virus with increased activity in the post monsoon months was concordant with reported patterns of dengue transmission, vertical transmission was observed mainly in summer months when human dengue infections were at lower level. In addition, most emergency control effects against Ae. aegypti are by insecticide space spraying and Ae. aegypti populations quickly rebound after spraying because larval habitats remain largely unaffected. Therefore, the simultaneous control of larvae and adults would be particularly important if there is vertical transmission of dengue viruses.

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