SHORT COMMUNICATION

Dengue Virus Type 3 Isolation from Aedes aegypti in the Municipality of Nova Iguaçu, State of Rio de Janeiro


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In a prospective field study conducted from July 2000 to June 2001, adult Aedes aegypti and Ae. albopictus mosquitoes were caught from the municipality of Nova Iguaçu, State of Rio de Janeiro, Brazil. Virus isolation in Ae. albopictus clone C6/36 cell line and a semi-nested reverse transcription-polymerase chain reaction detected only dengue virus type 3 in three pools of Ae. aegypti, despite the co-circulation of DEN-1, DEN-2 and DEN-3 serotypes in that area. No viruses were detected in Ae. albopictus mosquitoes. This virological surveillance consists in a sentinel system alerting for dengue outbreaks.

Key words: dengue - Aedes aegypti - Aedes albopictus - reverse transcription-polymerase chain reaction - virus isolation - Rio de Janeiro - Brazil

Dengue fever is an acute, mosquito-transmitted viral disease caused by any of four virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). Nowadays dengue is the most important human disease caused by arbovirus in the world. Its incidence has increased in tropical areas where the main vector Aedes aegypti (Linnaeus) has spread due to unplanned urbanization and lack of effective control (Gubler & Clark 1996).

In the last 16 years in Brazil an increase in the frequency of epidemics and the geographic expansion of both the mosquito vector and the viruses resulted in the co-circulation of DEN-1 and DEN-2 viruses in 25 out of 27 Brazilian states (Nogueira et al. 1999, Funasa 2001).

In the municipality of Nova Iguaçu, located in the Metropolitan Region of the State of Rio de Janeiro, dengue cases have been reported since DEN-1 virus was isolated for the first time in the State (Schatzmayer et al. 1986, Miagostovich et al. 1993). By the end of December 2000, DEN-3 virus was first isolated from an autochthonous case of dengue fever (Nogueira et al. 2001) when the house infestation levels of Ae. aegypti and Ae. albopictus were 8.1% and 4.5%, respectively (WC Silva, pers. commun.). Braga et al. (2000) observed that local samples of Ae. aegypti presented 58% of resistance to temephos.

The high dengue viruses activity in Nova Iguaçu stimulated a series of field studies aiming to elucidate several aspects of dengue vectors’ biology started in 1997 (Honório 1999, Honório & Lourenço-de-Oliveira 2001). Concomitantly with this field work, a project of virological surveillance has been carried out for the detection of dengue virus from field collected Ae. aegypti and Ae. albopictus (Skuse). From July 2000 to June 2001 adult mosquitoes were caught in 35 districts of that municipality. The captures were performed twice a week, alternately in the morning and in the afternon, with manual and battery backpack aspirators and with nets, both indoors and in the yards and gardens, close to the dwellings. Mosquitoes were captured while flying, seeking for blood or hiding in resting places. Mosquitoes were identified to species, pooled according to sex, date, district and stored in liquid N2 at the same day of collection.

From a total of 2,164 mosquitoes, 503 Ae. aegypti (352 females + 151 males) and 80 Ae. albopictus (58 + 22) were pooled (9-17 mosquitoes/pool) and processed for virus isolation. Briefly, mosquito pools were grounded in 1 ml of Leibovitz L-15 (Gibco BRL, Life Technologies) tissue culture medium and centrifuged for 15 min at 6,000 rpm. After treatment with penicillin-streptomycin – 10,000 units – (Gibco BRL, Life Technologies) 50 µl of the supernatants were inoculated into the monolayer of Ae. aegypti clone C6/36 cell line (Igarashi 1978) containing nine female mosquitoes each. All positive pools were confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) performed according to Lanciotti et al. (1992). This happened despite the inhibitory factors expected when RT-PCR is applied to mosquito pools. No dengue virus was detected from Ae. albopictus. Despite of the co-circulation of DEN-1, DEN-
2 and DEN-3 in Nova Iguaçu, only DEN-3 was isolated from mosquitoes. This was probably because mosquitoes were collected essentially in or close to houses reported to have human dengue cases, several of which latter confirmed to be due to DEN-3.

The positive mosquitoes were collected in the districts of Santa Eugênia, Califórnia and Morro Agudo on April 23rd, April 24th, May 28th respectively. Human cases due to DEN-3 virus were confirmed in the two last districts with onset of symptoms on March 3rd and April 27th, being Califórnia the district where the first DEN-3 virus was first isolated from an autochthonous case in Brazil (Nogueira et al. 2001). In 1998 DEN-3 virus had been isolated from an imported dengue case in São Paulo and no more cases were reported afterwards (Rocco et al. 2001). Nevertheless, the isolation of DEN-3 virus in female of Ae. aegypti together with the increasing number of DEN-3 laboratory diagnosed cases (91 by October 2001) confirmed the transmission of this serotype in the State of Rio de Janeiro.

Virological and entomological surveillances by detecting dengue infected mosquitoes in the field have been useful as an early warning monitoring system for dengue outbreaks in endemic areas and for the detection of new virus serotype invasion (Chow et al. 1998, Kow et al. 2001). Although DEN-3 virus has been detected in humans from Nova Iguaçu since December 2000, all mosquitoes collected and examined from July 2000 to March 2001 (287 Ae. aegypti and 78 Ae. albopictus) were negative. The three positive pools for DEN-3 were composed by mosquitoes caught in April and May 2001, just after the rainy season in the area. If we take into account that the local human population was susceptible to DEN-3 and that a female of both Ae. aegypti and Ae. albopictus may fly at least 800 m in 6 days in that area (Honório 1999), a rapid spreading of the virus was expected. However, it seems that, even if DEN-3 occurred in the area before December 2000, its transmission would become important only by the end of the rainy season (Jan-Mar) of 2001, when the local frequency of both mosquito species has the annual peak (Honório & Lourenço-de-Oliveira 2001). Indeed, after virus isolation from mosquitoes, the number of human cases by DEN-3 has slowly increased during the dry season of 2001 in Nova Iguaçu. But in the following rainy season, this virus has quickly spread in the State of Rio de Janeiro and lead to a severe outbreak of dengue fever and dengue hemorrhagric fever in 2002. These data emphasize the virological and entomological surveillances as sentinel systems alerting for dengue outbreaks.

This is the first report of DEN-3 virus isolation from pools of Ae. aegypti in the country. In 1986 DEN-1 was also isolated from three pools of Ae. aegypti mosquitoes collected in the same municipality of Nova Iguaçu (Nogueira et al. 1988). Other dengue virus serotypes have already been isolated from Ae. aegypti in other states of Brazil (Degallier et al. 1996).

The molecular characterization of the DEN-3 virus from Nova Iguaçu showed that it belongs to the same genotype of the DEN-3 strains circulating in American continent (MP Miagostovich, pers. commun.). These data stress the role of the State of Rio de Janeiro as an important entrance point of dengue viruses in Brazil since the introduction of DEN-1 and DEN-2 viruses in 1986 and 1990, respectively, which resulted in a rapid spread of those virus all over the country (Nogueira et al. 1999).

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REFERENCES


