

SHORT COMMUNICATION

Dengue Virus Type 3 in Rio de Janeiro, Brazil

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Dengue virus type 3 was isolated for the first time in the country as an indigenous case from a 40 year-old woman presenting signs and symptoms of a classical dengue fever in the municipality of Nova Iguaçu, State of Rio de Janeiro. This serotype has been associated with dengue haemorrhagic epidemics and the information could be used to implement appropriate prevention and control measures. Virological surveillance was essential in order to detect this new serotype.

Key words: dengue virus type 3 - virological surveillance - Brazil

Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are considered the most medically important and widespread mosquito-borne viral diseases. Dengue (DEN) viruses belong to the family *Flaviviridae*, genus *Flavivirus*, and occur as four antigenically related but distinct serotypes designated DEN-1, 2, 3 and 4 (Westaway et al. 1985). Although all dengue serotypes are responsible for both DF or DHF/DSS, dengue subtyping methods have been used for epidemiologic and pathogenesis investigations, since genetic differences could reveal important information related to the disease (Lanciotti et al. 1994, Rico-Hesse et al. 1997).

In Brazil, since 1986 dengue epidemics have been occurring annually, becoming a national public health problem. A total of 1,832,371 cases were reported, resulting from DEN-1 and/or DEN-2 epidemics, and occurred in 24 out of 26 states and the Federal District/Brasília (Ministry of Health, Brazil). Molecular analysis performed on genome fragments from DEN-1 and DEN-2 viruses identified the Caribbean and Jamaican genotypes, respectively, as the virus strains circulating in the country (Rico-Hesse 1990, Vorndam et al. 1994, Miagostovich et al. 1998). The Jamaica genotype

introduction into the Americas resulted in both an increase in the severe forms of the disease and epidemics in which there were a significant number of DHF/DSS cases (Nogueira et al. 1993, Rico-Hesse et al. 1997).

In January 2001, during a virological surveillance carried out in the municipality of Nova Iguaçu, DEN-3 was isolated from a 40 year-old woman presenting signs and symptoms of a classical DF (WHO 1986). This municipality is located 25 km away from Rio de Janeiro downtown in greater Rio and was the same municipality where DEN-1 was first isolated in 1986 (Schatzmayr et al. 1986). Nova Iguaçu has reported subsequent epidemics of DF, having DEN-1 and DEN-2 viruses isolated during the summer 2000-2001.

DEN-3 virus isolation was performed by inoculation of serum sample into clone C6/36 *Aedes albopictus* cell line and typed by the indirect fluorescent antibody test using serotype-specific monoclonal antibodies (Gubler et al. 1984). Viral RNAs were extracted from the supernatants of infected cells and from sera using a QIAamp Viral Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. The nested RT-PCR (Lanciotti et al. 1992) detected DEN-3 directly from serum sample and confirmed DEN-3 from tissue culture fluid (Figure).

Serological results showed IgM seroconversion by IgM capture enzyme-linked immunosorbent assay (Kuno et al. 1987) while G-ELISA (Miagostovich et al. 1999) characterized dengue primary infection showing titers of <40 and 1/2560 on the acute and convalescent serum, respectively.

The above-mentioned data demonstrate the importance of on-going monitoring of samples of DEN viruses circulating in the country in order to

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Characterization of DEN-3 virus by reverse transcriptase-polymerase chain reaction (RT-PCR). Ethidium bromide 1% stained agarose gel showing RT-PCR nested products: lane 1, 100-bp ladder (Gibco); lane 2 molecular weight marker of DEN-1 and DEN-2 RT-PCR products corresponding to 482bp and 119bp, respectively; lanes 3 and 7, DEN-3 from fluid culture of *Aedes albopictus* clone C6/36 infected with serum sample and serum sample from the same patient, respectively; lanes 4, 5 and 6 negative sera; lanes 8 and 9, DEN-1 and DEN-2 from sera; lanes 10, 11 and 12 positive controls of DEN-3, DEN-1 and DEN-2; lane 13 water

identify the entry of a new serotype or genotype and a potential association between genotype and virulence. The introduction of this new DEN serotype, with a possibility of a large outbreak may represent a new threat, since DEN-3 virus has been associated with the dengue haemorrhagic occurred in India, Sri Lanka and Central America (Lanciotti et al. 1994, Anonymus 1995).

Three more DEN-3 viruses were isolated afterwards in the State of Rio de Janeiro, two of them in the same area of the first isolated strain.

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