Short Communication

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Identification of dengue viruses in naturally infected *Aedes aegypti* females captured with BioGents (BG)-Sentinel traps in Manaus, Amazonas, Brazil

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ABSTRACT

Introduction: In Manaus, the first autochthonous cases of dengue fever were registered in 1998. Since then, dengue cases were diagnosed by the isolation of viruses 1, 2, 3, and 4. **Methods**: One hundred eighty-seven mosquitoes were collected with BioGents (BG)-Sentinel traps in 15 urban residential areas in the Northern Zone of Manaus and processed by molecular tests. **Results**: Infections with dengue viruses 1, 2, 3, and 4 and a case of co-infection with dengue viruses 2 and 3 were identified. **Conclusions**: These findings corroborate the detection of dengue in clinical samples and reinforce the need for epidemiological surveillance by the Health authorities.

Keywords: *Aedes aegypti*. Dengue. Virus. Molecular tests.

Manaus, Amazonas (AM) was infested by *Aedes aegypti* in 1996 and the first autochthonous cases of dengue fever were registered in March 1998[1]. Since then, dengue fever cases were diagnosed in the following years with the isolation of dengue viruses (DENV) 1, 2, 3, and 4 in 2008[2]. In the present work, all 4 serotypes of the virus were detected in naturally infected *A. aegypti* by reverse transcription-polymerase chain reaction (RT-PCR), the amplicon sequence of DENV-4 was analyzed, and these findings were corroborated with the detection of DENV-4 in clinical samples of patients residing in the northern zone of Manaus, AM.

A total of 187 mosquitoes were collected with BioGents (BG)-Sentinel traps[3] from January 2009 to June 2010 in 15 urban residential areas in the northern zone of Manaus, AM, Brazil. Sixty BG-Sentinel traps (4 traps in each of the 15 areas) were installed in each area every 2 weeks for a 24-h time period. The captured mosquitoes were identified and sexed. Cephalothoraxes of *A. aegypti* were separated for each area and each collection date in 2-mL Eppendorf cups (pool size: 1-10 mosquitoes) and maintained at -70°C until analysis.

The pooled samples were pulverized and macerated on ice with 300mL Trizol reagent (Invitrogen,Brazil). Viral RNA extraction was performed according to the manufacturer's instructions and reverse transcription was performed using Superscript III (Invitrogen, Brazil) and random primers. Based on the previously described protocol[4], semi-nested PCR was conducted to amplify a region of the viral genome that contains the capsid-premembrane (C-prM) genes, with primers designed to amplify 119-bp DENV-2, 290-bp DENV-3, and 392-bp DENV-4. The generated amplicon of DENV-4 was sequenced in both directions using the BigDye Terminator cycle sequence kit (Applied Biosystems, Foster City, CA, USA).

Of the total of 187 pools of *Aedes aegypti* that were processed between August 2009 and July 2010, which each contained 10 females, 1 pool was positive for DENV-1, 4 pools were positive for DENV-2, 3 were positive for DENV-3, and 2 were positive for DENV-4. One pool was co-infected with DENV-2 and 3. The obtained sequence DENV-4 was submitted to a basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov.entrez) analysis, which uses the megablast algorithm optimized for highly similar sequences. By using this approach, sequences of DENV-4 showed a similarity of 98% with the standard sequence H241-P, which represents genotype I of DENV-4. A phylogenetic tree **(Figure 1)** was generated using neighbor-joining analysis with molecular evolutionary genetics analysis (MEGA) 4[5]. The nucleotide sequence from *A. aegypti* was deposited in GenBank under the accession number HQ840706.

Genotype I of DENV-4, which is often associated with cases of dengue hemorrhagic fever, is believed to be present only on the Asian continent. This genotype was previously identified in strains from Manaus, which were deposited in Genbank

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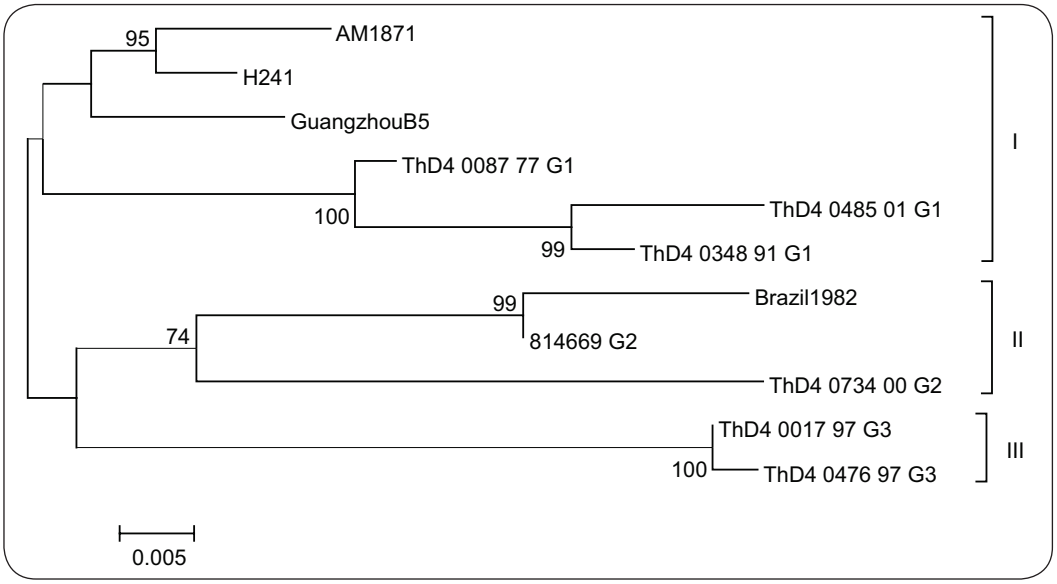

FIGURE 1 - Phylogenetic tree of dengue type 4 sequence references and the sequence from *Aedes aegypti* mosquitoes obtained in Manaus, Amazonas, Brazil. The tree was generated using neighbor-joining analysis with MEGA 4. MEGA: molecular evolutionary genetics analysis.

in 2008[6]. The presence of this genotype in Brazil, especially Manaus, could be explained by existing economic relationships between the industrial pole of Manaus and Asian countries. To date, no dengue fever epidemic caused by DENV-4 has occurred in Brazil. Studies suggest that the introduction of a DENV to an area with a population that is susceptible can immediately lead to an epidemic[7].

The presence of DENV has been previously detected in *A. aegypti* and other mosquito species[8], and DENV co-infections have been reported in clinical samples[9]. These findings of the present study in *A. aegypti* mosquitoes corroborate the detection of DENV in clinical samples and reinforces the need for strengthened epidemiological surveillance of dengue infection by the Health authorities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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