

## VERTICAL TRANSMISSION OF DENGUE VIRUS IN *Aedes aegypti* COLLECTED IN PUERTO IGUAZÚ, MISIONES, ARGENTINA

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### SUMMARY

A finding of vertical transmission of the DEN 3 virus in male specimens of *Aedes aegypti*, collected in the 2009 fall-winter period, in Puerto Iguazú city, Misiones, Argentina, using the RT-PCR technique in a 15-specimen pool is reported. This result is analyzed within the context of the epidemiological situation of Argentina's northeast border.

**KEYWORDS:** Dengue; *Aedes aegypti*; Vertical transmission; Argentina.

### INTRODUCTION

Transovarial transmission of the dengue virus, which was found in non-hematophagous male specimens, or in the aquatic stage of larvae and pupae, was reported early in the 1950s by ALBERT SABIN<sup>21</sup>, in a pioneering piece of work on different aspects of the disease and subsequently, in the 1980s, on *Aedes aegypti* and *Aedes albopictus* by different researchers<sup>9,11,19,20</sup>. In recent decades, research has increased, some of it originating in clinical laboratory tests in which mosquitoes were infected<sup>3,10</sup>, and others originating in field studies which accounted for natural transmission. The latter observations come both from researchers from the Americas<sup>8,9,14</sup>, especially Brazil<sup>4,6,15,16,23,24</sup>, India<sup>1,2</sup> and South East Asia<sup>5,12,17</sup>. The four dengue virus serotypes were found with natural vertical transmission. In India, higher transovarial transmission rates were found during winter for *A. albopictus*, whereas, *A. aegypti* showed higher rates in warm and rainy seasons in arid or semi-arid areas<sup>1</sup>. In another work, simultaneous vertical transmission for DEN 2 and DEN 3 was found in *A. albopictus*<sup>16</sup>.

This form of transmission could be relevant to keep viral circulation running during interepidemic periods. However, the role that the occurrence of outbreaks can have is unknown, with the typical form of transmission being: person to viremia- vector- susceptible person, being the most important one.

### MATERIALS AND METHODS

The collections were made in Puerto Iguazú city, located at 25° 36' south latitude and 54° 35' west longitude, in the province of Misiones, Argentina. With an overall surface of 760 km<sup>2</sup> and a population of 82,227 inhabitants (2010 census), the city is set up in the tri-border

area, opposite Foz do Iguazú city in Brazil and Ciudad del Este in Paraguay (Fig. 1).



Fig. 1 - Georeferencing of Puerto Iguazú city, Misiones, Argentina.

During the period ranging from April to September 2009, adult mosquitoes were collected in the urban area of the city, using BG-Sentinel<sup>®</sup> traps. Specimens were classified by genus and species and separated by sex to constitute 10 pools of 15 to 20 mosquitoes. The ratio between the number of positive pools and the total number of mosquitoes studied, multiplied by one thousand, is the minimum infection rate (MIR).

RNA extraction was made using the Trisol LS Reagent<sup>®</sup> method (Invitrogen<sup>™</sup>) according to the manufacturer's protocol. RT-PCR was performed on the mosquito pool according to LANCIOTTI *et al.* protocol<sup>13</sup>. The primers used in the first round were:

D1 (5'-TCAATATGCTGAAACGCGCGAGAAACCG-3') and D2 (5'-TTGCACCAACAGTCAATGCTTCAGGTC-3') of Operon<sup>™</sup>.

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A nested-PCR was performed in the second round with D1 primer and those specific for each dengue serotype (TS1, TS2, TS3 and TS4) which amplify the genC-prM regions in: 482, 119, 290 and 392 base pairs of DEN 1, DEN 2, DEN 3 and DEN 4 respectively. Detection of amplified fragments was done with 1.5% agarose gels revealed with GelRed®. The amplicon obtained was purified and sequenced by the company Macrogen™, Seoul, Korea. The nucleic acid sequence was compared with sequences of the GenBank BLAST, using the Clustal W (Megalalign Software) alignment program.

## RESULTS

For male specimens of *Aedes aegypti*, only one 15-mosquito pool could be constituted, which proved positive for DEN 3. Sequencing of nucleic acids (Fig. 2) compared with sequences of the GenBank yielded 100% compatibility with sequences (ID): EF546774.2, EF546773.2, FJ373306.1, FJ182005.1, AB038479.1 and resulted in the following reports:

### Lineage Report

Dengue virus

Dengue virus 3. 55 295 hits [viruses] Dengue virus type 3 isolate INDI06DEN13 polyprotein gene, p.

Dengue virus 2. 55 2 hits [viruses] Dengue virus type 2 isolate 152/BRAZ/99 nonfunctional polyp.

### Taxonomy Report

Dengue virus. 297 hits, 2 orgs [root; Viruses; ssRNA viruses; ssRNA positive-strand viruses; Flaviviridae; Flavivirus; Dengue virus group]

Dengue virus 3. 295 hits, 1 orgs.

Dengue virus 2. 2 hits, 1 orgs.

Taking into account that the sample was a very small one, MIR was not calculated.

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ATGCTTCGACATGGTGGTGGACTCAGGATGCTCTGTCTCATGAT
GATGTTAAAAATGCCTCCCTACCGGACGTCATGATGCGACCT
CCCTCCATCGTTAATCCTCCTGACTGACCTATACTGAACAGTA
CCTTGAGGGAAAGGCAGAAAGAACCCTGGCGAGGGGAGTGAA
ACAGAACCCTGAAGACATGACTGTTGGTGCAAGCTCTGTCTCA
TGATGATGTTAAAGAAGGAAAAAATTACGCCCATGCCCAAGG
GAAAACCCCACTTTAAAGTATCCCTCTCGTGGAAAGGCCAATAT
CTGCGCGAAAAATTGCTTTTGTGCTTCCCAAGATCCCGTTCTG
TACTCTGCAGAGATGTGCATGTGGACAACGTGTTGIAGGGGGA
TGGTCTGTCAACACCCCCACCTCGTGAGGCACCTCCCGCCAT
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Fig. 2 - Nucleotide sequence of the isolated fragment. ADN transcript (434 pb).

## DISCUSSION

This is the first communication of vertical transmission of the dengue virus in *Aedes aegypti* in Argentina. Although there is a lack of knowledge regarding the frequency with which it can occur, the degree of importance of the genesis and evolution after an outbreak, or whether it can also originate it *per se*, it is important to consider that it has been detected in a region with intense circulation of people due to the high levels of tourist traffic that occurs there throughout the year. DEN 3 serotype was the cause of an outbreak of serious magnitude in Paraguay, between December 2006 and May 2007, and which had an impact on Argentine cities near border areas. In 2000, the northeast region had presented DEN 1 cases associated to the Paraguay outbreak (December

1999 to May 2000). On the other hand, in 2009, dengue showed very low activity in the northeast border; this was due to DEN 2. In the same year, Argentina had the highest record of cases in history as a result of the epidemic, which originated in the northeast and which also affected big cities, including Buenos Aires, due to DEN 1<sup>22</sup>. As of 2011 and until this article was written (January 2013), the dengue patients assisted by our service and who came from Paraguay correspond to DEN 2, the serotype that had not had any epidemic circulation in the region until said year.

The finding of DEN 3 with vertical transmission in *Aedes aegypti*, in situations in which other serotypes are circulating, could constitute a natural reservoir, also taking into account that the population's immunity against DEN 3 should be high, as a result of the cases that occurred in 2007.

An important aspect, which has recently been reported, is the finding of *Aedes albopictus*<sup>6</sup> in Puerto Iguazú, Misiones, which is the other dengue vector with proven capacity for vertical transmission.

In this work, mosquitoes were collected during the fall-winter months, which do not record occurrences of cases. This fact can strengthen the hypothesis in which vertical transmission would keep viral circulation running during interepidemic periods, but other studies relate it to the pre-epidemic period<sup>1,14</sup>.

Due to the fact that the sample was reduced, no indices could be established or statistical analyses carried out; however, it is surprising that only one pool with 15 specimens studied has been positive. On the other hand, a study carried out in Colombia<sup>18</sup> did not demonstrate vertical transmission in one sample of 1,400 male *A. aegypti* specimens, when the MIR in over two thousand female specimens was 11.6%.

Circulation of the three serotypes, the presence of two *Aedes* species with transmission capacity and the finding of vertical transmission make the Argentine northeast an area of high risk for dengue occurrence.

Within this line of investigation, it is necessary to increase the number of collections at throughout the year, which will improve sensitivity to entomological surveillance, enable estimation of temporal-spatial variations and improve knowledge about vertical transmission in each species.

## RESUMEN

### Transmisión vertical de virus dengue en *Aedes aegypti*, capturados en Puerto Iguazú, Misiones, Argentina

Se comunica el hallazgo de transmisión vertical de virus DEN 3 en ejemplares machos de *Aedes aegypti*, capturados en otoño-invierno de 2009, en la ciudad de Puerto Iguazú, Misiones, Argentina, utilizando la técnica de RT-PCR en un pool de 15 ejemplares. Se analiza este resultado en el contexto de la situación epidemiológica de la frontera nordeste de Argentina.

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