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# First Record of Natural Transovarial Transmission of Dengue Virus in *Aedes albopictus* from Cuba

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*Abstract.* Transovarial transmission (TOT) of dengue virus (DENV) in *Aedes* spp. is an important mechanism for DENV maintenance in nature and may be important in initiating outbreaks. The objective of this study was to explore the occurrence of TOT in wild *Aedes albopictus* populations in Cuba. Mosquito larvae were collected in Cotorro municipality, Havana, Cuba, and identified to species. Fifteen pools of *Ae. albopictus* each containing 30 larvae were processed for DENV detection by using conventional reverse transcription polymerase chain reaction (RT-PCR) and nested PCR. Four out of 15 pools processed were positive for DENV-3, but no other DENV serotype was detected. This is the first time TOT of DENV detected in Cuban field populations of *Ae. albopictus*, and this suggests that this species may be an important vector of DENV in Cuba.

The world has experienced an increase in dengue incidence in the recent years. Recent investigations estimate that approximately 2.5 billion people live in countries where dengue fever is endemic and the WHO estimates 100–400 million infections every year.[1] Dengue illness is distributed worldwide and is mainly transmitted by two widespread mosquito vectors *Aedes* (*Stegomyia*) *aegypti* (L) and *Aedes* (*Stegomyia*) *albopictus* (Skuse). Dengue is caused by dengue virus (DENV) that has four different serotypes (DENV-1 to DENV-4).[2]

In Cuba, the first dengue epidemic was reported in 1977–1978 caused by DENV-1. Subsequently, the country's first hemorrhagic dengue epidemic was reported in 1981, caused by DENV-2, which stopped after an intense vector control campaign that kept the country free of transmission for 14 years.[3] But the eastern part of the country suffered another epidemic of dengue hemorrhagic fever in 1997 that lasted 6 months[3] and was caused by the same serotype. In 2000, a limited outbreak of DENV-4 was reported in Havana, resulting in 138 confirmed cases.[4] In June 2001, the nationwide dengue case surveillance system identified DENV-3 circulating in Havana that eventually expanded to involve 12,889 serologically confirmed cases, including 78 dengue hemorrhagic fever/dengue shock syndrome and three fatalities.[5] Unfortunately, Cuba has been reporting annual dengue outbreaks since 2006.[6]

*Aedes aegypti* is the main vector of dengue in the world. *Aedes albopictus* is considered the potential vector of this arbovirus in some regions where *Ae. aegypti* is absent such as Southeastern Asia, Europe, and the Mediterranean.[7] However, *Ae. albopictus* has never been incriminated in the horizontal transmission of DENV in the American region despite having reported vertical transmission in a dengue outbreak that affected Mexico[8] and despite detecting the virus in adult mosquitoes collected in Brazil.[9]

*Aedes albopictus* was introduced in Cuba in 1995,[10] and currently it is distributed in 14 of the 15 provinces of the country.[11] However, the role of *Ae. albopictus* in the transmission of DENV in Cuba is unknown.

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The study was carried out in the municipality of Cotorro de La Habana (23° 01′34″ N, 82° 14′51″ W), Cuba, in November 2019. The area is divided into three health jurisdictions (Cuatro Caminos, Efraín Mayor, and Rafael Valdés), with a total area of = 65.9 $km^2$ and a population density of 1,219.8 inhabitants/$km^2$. *Aedes albopictus* larvae were collected by workers from the National *Ae. aegypti* Control Program in the town of Cuatro Caminos. A total of 450 mosquito larvae were morphologically identified as *Ae. albopictus* at the Vector Control Department of the Pedro Kourí Institute of Tropical Medicine (IPK). The mosquito larvae were collected from 15 tree holes scattered in an area of = 100 $m^2$ and grouped into pools of 30 larvae for DENV screening. Larvae were homogenized using a Tissue Lyser homogenizer (QIAGEN, Hilden, Germany) at a frequency of 30 cycles per minute, centrifuged at 4°C to 20,000 × g for 5 minutes, and 140 μL of the supernatant was processed for RNA isolation using the QIAamp Viral RNA Mini kit (QIAGEN) according to the manufacturer's protocol. RNase P was used as an internal control in each reaction.

Detection of DENV serotypes was by using conventional RT-PCR in 50-μL volume reactions, where 10 μL of RNA were amplified using the OneStep RT-PCR kit (QIAGEN) and a nested PCR was subsequently performed adding 2 μL of PCR product from the initial amplification with GoTaq DNA polymerase Kit (Promega, Madison, WI). Both reactions used deoxynucleotide triphosphates and primers, as described by Lanciotti.[12] The length of the PCR products was visualized on a 2% agarose gel. RNA of the following dengue strains was used as positive controls: DENV 1 Hawaii, DENV 2 New Guinea C, DENV 3 H-87, and DENV 4 H-241.

The minimum infection rate (MIR) was calculated using the standard formula: MIR = (number of positive pools/total number of larvae tested) x 1,000.[13]

DENV-3 serotype was detected in four out of 15 pools of *Ae. albopictus* larvae from Cotorro municipality (Figure 1A and B) and the MIR was 8.88. No other DENV serotypes were detected in any of the pools.

This is the first evidence of DENV TOT in *Ae. albopictus* populations in Cuba. Previous studies detected all four DENV serotypes in the immature stage of *Ae. aegypti*[14] suggesting widespread TOT of DENV in this species. These results are consistent with studies in northern Mexico where DENV-2

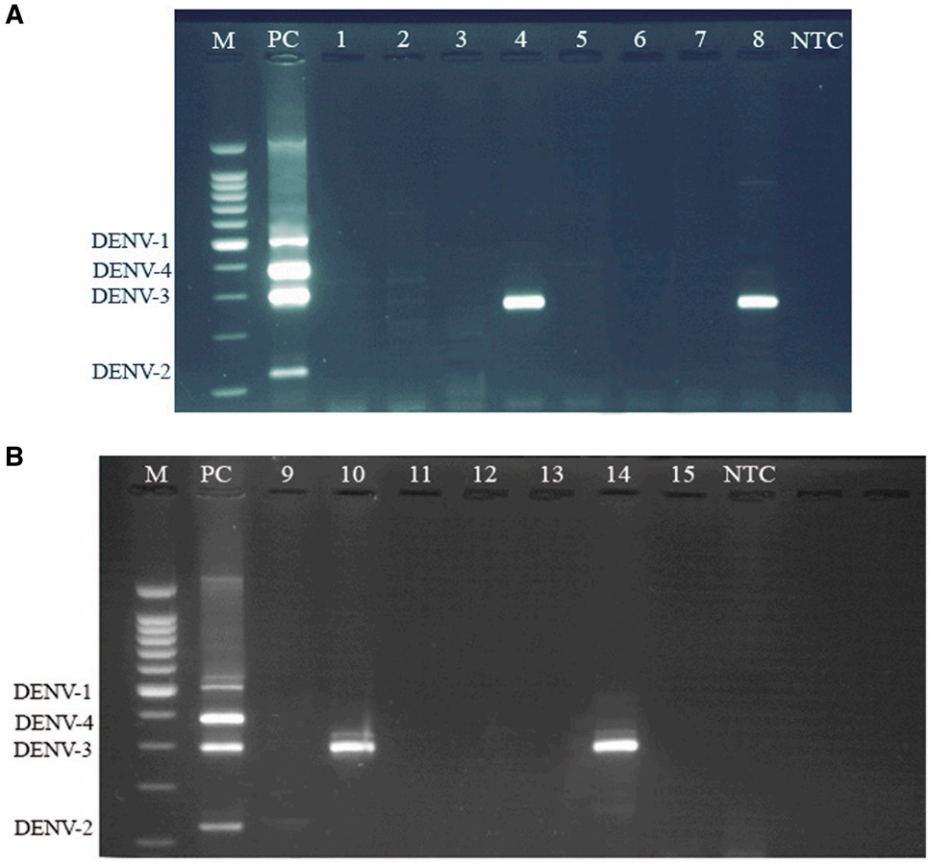


FIGURE 1. (**A, B**) Nested RT-PCR assay of DENV in pools of *Ae. albopictus* larvae. Positive control (PC) of DENV serotypes. (**A**) Lanes 4 and 8 showed the products of DENV-3 (290 bp) obtained by nested PCR following RT-PCR. (**B**) Lanes 10 and 14 showed the products of DENV-3 (290 bp) obtained by nested PCR following RT-PCR. Non-template control (NTC) and lane M, marker of 100 bp. DNA sizes are given in base pairs.

and DENV-3 were detected in pool of 10 field-collected *Ae. albopictus* males.[8] In Costa Rica, DENV was detected in a pool of field-collected *Ae. albopictus* males.[15] Studies in Brazil detected DENV-1 and DENV-3 in the immature stages of *Ae. albopictus*[16] and DENV-1 and DENV-4 in adults.[9]

In the present study, the estimated MIR of 8.8 is less than that reported during a dengue outbreak in Tokyo, Japan (MIR of 21.3),[17] but greater than the MIR (0.7) reported in Indonesia.[18]

The finding of DENV-3 RNA in Cuban *Ae. albopictus* larvae strongly suggests that vertical transmission from infected female to offspring occurred, which could have an important implication to the epidemiologic surveillance system of dengue. Some authors pointed out that the vertical transmission of DENV can be detected either during an epidemic or months before an outbreak.[19] Other researchers hypothesize that *Aedes*-infected eggs can persist for several months and hatch when environmental conditions are favorable leading to the emergence of infected females that could initiate horizontal transmission of dengue without having previously bitten infected human hosts. A recent study detected Zika virus in the salivary glands of a vertically infected *Ae. aegypti* female suggesting that TOT frequently occur in the field.[20] Although some studies have proposed monitoring of DENV in *Aedes* larvae to predict dengue outbreaks, none of them have showed a statistical association between the detection of DENV in larvae and dengue outbreaks.[21] *Aedes albopictus* has not ever been incriminated in the transmission of dengue in Cuba, even though it is recognized as primary vector of this arbovirosis in some Asian and European countries[17,22] and its vector competence to dengue and other arbovirosis has been demonstrated in the laboratory.[23] Our results strongly suggest that vector competence and TOT studies of Cuban populations of *Ae. albopictus* for DENV can help elucidate the potential role of *Ae. albopictus* as a DENV vector in Cuba. This may lead to modifications of vector control strategies in Cuba to combat *Ae. albopictus* because of the different ecological habits of *Ae. albopictus* compared with *Ae. aegypti.*

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