

Occurrence of Natural Vertical Transmission of Dengue-2 and Dengue-3 Viruses in *Aedes aegypti* and *Aedes albopictus* in Fortaleza, Ceará, Brazil

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Abstract

Background: *Aedes aegypti* and *Aedes albopictus* perform an important role in the transmission of the dengue virus to human populations, particularly in the tropical and subtropical regions of the world. Despite a lack of understanding in relation to the maintenance of the dengue virus in nature during interepidemic periods, the vertical transmission of the dengue virus in populations of *A. aegypti* and *A. albopictus* appears to be of significance in relation to the urban scenario of Fortaleza.

Methods: From March 2007 to July 2009 collections of larvae and pupae of *Aedes* spp were carried out in 40 neighborhoods of Fortaleza. The collections yielded 3,417 (91%) *A. aegypti* mosquitoes and 336 (9%) *A. albopictus* mosquitoes. Only pools containing females, randomly chosen, were submitted to the following tests indirect immunofluorescence (virus isolation), RT-PCR/nested-PCR and nucleotide sequencing at the C-prM junction of the dengue virus genome.

Results: The tests on pool 34 (35 *A. albopictus* mosquitoes) revealed with presence of DENV-3, pool 35 (50 *A. aegypti* mosquitoes) was found to be infected with DENV-2, while pool 49 (41 *A. albopictus* mosquitoes) revealed the simultaneous presence of DENV-2 and DENV-3. Based on the results obtained, there was a minimum infection rate of 0.5 for *A. aegypti* and 9.4 for *A. albopictus*. The fragments of 192 bp and 152 bp related to DENV-3, obtained from pools 34 and 49, was registered in GenBank with the access codes HM130699 and JF261696, respectively.

Conclusions: This study recorded the first natural evidence of the vertical transmission of the dengue virus in populations of *A. aegypti* and *A. albopictus* collected in Fortaleza, Ceará State, Brazil, opening a discuss on the epidemiological significance of this mechanism of viral transmission in the local scenario, particularly with respect to the maintenance of these viruses in nature during interepidemic periods.

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Introduction

Dengue is a mosquito-borne infection that in recent decades has become a major international public health problem, mainly in tropical and subtropical areas [1]. The World Health Organization estimates that 50–100 million people are infected annually with the dengue virus (DENV) worldwide [2]. In Brazil, the first isolated serotype (DENV-1) was in the state of Roraima in 1981; however, in 1986, when this serotype was reintroduced in Brazil, dengue became a major public health problem, and by 2009 more than 5.1 million cases of dengue had been reported [3]. In the same period 389,016 cases of dengue fever were reported in the

state of Ceará (northeastern Brazil), of which 42% were recorded in the city of Fortaleza [4]. Between 2007 and 2009, it was identified the circulation of DENV-1, 2 and 3 in Brazil, while in the state of Ceará and Fortaleza city there were identified DENV-2 and 3. In the same period, there were almost 50,000 cases of dengue in Fortaleza and 1.4 million cases in Brazil (Table 1).

The dengue virus belongs to the family Flaviviridae, genus Flavivirus, which are phylogenetically related to other important human pathogens, such as the yellow fever (YFV), Japanese encephalitis (JEV), and West Nile (WNV) viruses. The virions are enveloped spherical particles with a single-stranded, positive-sense

Table 1. Dengue cases and DENV circulating in Brazil, Ceará and Fortaleza, during 2007 to 2009.

Year	Cases of dengue disease/Dengue virus serotype (DENV)		
	Brazil	Ceará	Fortaleza
2007	475,496/1, 2 and 3	34,359/2 and 3	11,260/2 and 3
2008	585,769/1, 2 and 3	44,244/2 and 3	34,109/2 and 3
2009	393,583/1, 2 and 3	5,144/2	4,210/2

Source: Brazil Health Surveillance Secretariat/Ceará State Health Secretariat/ Fortaleza Municipality Health Secretariat.
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RNA genome of around 11 kb containing a single open reading frame encoding a single polyprotein co- and post-translationally cleaved into 3 structural (C, prM and E) and 7 nonstructural (NS1, NS2A, NS2B, NS, NS4A, NS4B and NS5) proteins. There are 4 genetically distinct DENV types (DENV-1 to -4), with multiple genotypic variants [5,6].

Dengue is an arbovirolosis transmitted mainly by *Aedes aegypti* and *Aedes albopictus* [7]. *A. aegypti* is a tropical mosquito considered the main vector involved in the urban transmission cycle of the DENV [8]. In Brazil, *A. aegypti* has been responsible for dengue transmission since the early 1980s [9]. *A. albopictus* has adapted to both tropical and temperate climatic regions and has colonized several types of breeding sites in urban and suburban areas [10]. Since it was recorded in Brazil in 1986 [11], *A. albopictus* has not been associated with dengue epidemics in the country, although it has been found naturally infected with YFV and DENV. Laboratory studies have shown their potential to become infected and transmit 20 other arboviruses [12]. In Ceará state, the first record of its presence occurred in 2005 in Fortaleza city [13].

The best known mechanism of DENV transmission is horizontal transmission (human-mosquito); however, transovarial or vertical transmission, where the female-infected mosquito is able to transmit the virus to its progeny, may provide a mechanism to understand how DENV persists in nature in the absence of non-immune vertebrate hosts or under environmental conditions unfavorable for mosquito activity [14,15].

The current study reports the isolation of DENV-2 and DENV-3 viruses in C6/36 cell cultures of *A. albopictus* and by reverse transcription-polymerase chain reaction (RT-PCR) in pools of *A. aegypti* and *A. albopictus* collected directly from the field in an urban area in Fortaleza city, state of Ceará, Brazil, during the period of 2007 to 2009.

Methods

Collection, Maintenance of Immature Forms and Identification of Adult Forms of *Aedes spp*

The city of Fortaleza, capital of Ceará State, is located on the northern coast of the Northeast region of Brazil. With 313.14 km² of surface area and a demographic density of 7,851.27 inhabitants/km², it is currently the fourth largest state capital in terms of population, its total residential population, in 2008, being estimated at 2,458,545 [16]. Since 1997 it has been administratively organized into 6 Regional Executive Secretariats (*Secretarias Executivas Regionais* - SER), in which lie its 116 neighborhoods.

From March 2007 to July 2009, during the activities of the Dengue Control Program in Fortaleza, which are performed every three months, larvae and pupae of *Aedes spp* were collected in households located in 40 neighborhoods of the city (Figure 1).

There was no association between the choice of neighborhoods and dengue cases, which were selected according to their infestation rates (Table 2). Parks with a large areas of plant cover frequented by the population for leisure activities were also included in the research. The specimens were separated and transferred to special containers, according to their stage of development. The larvae were kept in plastic containers of 200 mL while the pupae were distributed in 500 mL plastic containers, which were placed inside a cage, adapted for the maintenance of the adult forms. Upon reaching the adult stage mosquitoes were fed only with a sucrose-based solution and, therefore they were completely deprived of having a blood meal. Finally, five days after emergence, adults were identified for the presence of *A. aegypti* and *A. albopictus* [17], while other species of Culicidae were discarded. After identification, mosquitoes were separated into pools of 1 to 50 specimens (according Oswaldo Cruz Foundation protocol's), by month of collection, and stored at -80°C.

Preparation of Mosquitoes Samples for Virus Isolation and Characterization

Were randomly selected 47 pools of *A. aegypti* and *A. albopictus* females which were macerated in 2 mL microtubes containing 1 mL of L-15 Leibovitz medium (Sigma-Aldrich), supplemented with 100 mL of 2,95% tryptose phosphate (Sigma-Aldrich), 10 mL of a solution of non-essential amino acids (Sigma-Aldrich), 10 mL of 2% L-glutamine (Sigma-Aldrich), and 3 µL of a combined solution of the antibiotics penicillin and streptomycin (10,000 U/mL penicillin G sodium +10,000 µg/µL of streptomycin sulfate in 0.85% saline - Gibco®). Samples were then centrifuged at 2,000×g (30 min at 4°C) and the supernatants were transferred to 1.5 mL microtubes containing 100 mL of penicillin/streptomycin and Amphotericin B (Gibco®) and kept in an ice bath for 2 h. After this period, the samples were centrifuged at 2,000×g (20 min at 4°C). Finally, the supernatants were transferred to new microtubes of 1.5 mL containing 0.3 mL of fetal calf serum (Laborclin®), which were kept at -80°C until virus isolation.

The macerated samples (150 µL) were inoculated into C6/36 cell cultures of *A. albopictus*, according to the protocol established by Igarashi [18]. After two passages (seven days each), cells were subjected to indirect fluorescent antibody test (IFA) using specific monoclonal antibodies for the four dengue virus serotypes, according to the protocol established by Gubler et al. [19]. Cultures of uninfected cells were used as negative controls.

RNA Extraction, Reverse-transcriptase Polymerase Chain Reaction (RT-PCR) and Nested-PCR

Viral RNA extraction was performed with 250 µL of each cell culture fluids, using the Trizol® LS Reagent (Invitrogen®) method, following the manufacturer's protocol. RT-PCR for detecting DENV in mosquito pools was performed according to Lanciotti et al. [20], with minor modifications. This technique was used to exclude possible laboratory contamination, and provide additional data for future studies on the degree of variation in the genomic segment used. The first step of RT-PCR consisted of a reverse transcription reaction to synthesize and amplify a 511 bp cDNA fragment from RNA templates, corresponding to the C-prM junction of the dengue virus genome. The consensus primers forward D1 (5'-TCAATATGCTGAAACGCGCGAGAAACCG-3'/Invitrogen®) and reverse D2 (5'-TTGCACCAACAGT-CAATGTCTTCAGGTC-3'/Invitrogen®), and SuperScript™ III (Invitrogen®) and Platinum® Taq DNA polymerase (Invitro-

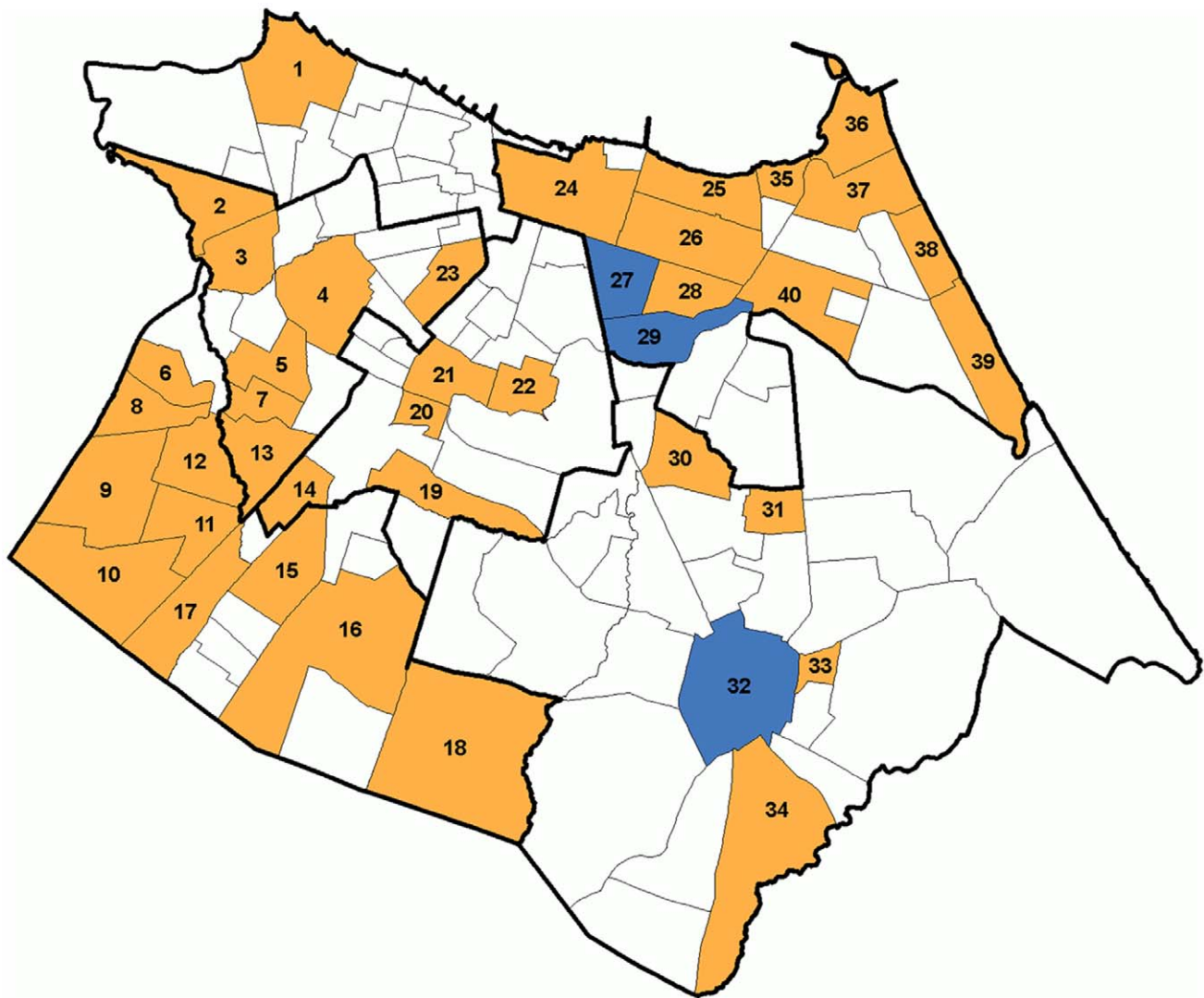


Figure 1. Neighborhoods of Fortaleza where collections of *Aedes spp* were carried out. DENV was isolated from *Aedes* mosquitoes in the neighborhoods colored in blue.
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gen[®] enzymes were used. The second step of the nested-PCR was carried out with D1 and type-specific (TS) reverse primers (TS1: 5'CGTCTCAGTGATCCGGGG3'; TS2: 5'CGCCA-CAAGGGCCATGAACAG3'; TS3: 5'TAACATCATCATGAGACAGAGC3'; TS4: 5'CTCTGTTGTCTTAAACAAGAGA 3'/Invitrogen[®]), which amplify regions of 482, 119, 290 and 392 bp of DENV-1, DENV-2, DENV-3 and DENV-4, respectively. The detection of amplified fragments was performed by gel electrophoresis (1.5% agarose gel stained with 1% ethidium bromide).

Estimation of Minimum Infection Rate (MIR)

The minimum infection rate (MIR) of the mosquito pools was calculated from the ratio between the number of positive pools and the total number of mosquitoes tested, multiplied by 1000 [21].

Nucleotide Sequencing

The PCR products were purified using the Invitrogen Pure-Link[™] kit and sequenced in an ABI Prism 3100 (Applied Biosystems) using a Big Dye Terminator 3.0 kit (California, U.S.)

and D1 (forward), TS-2 and TS-3 (reverse) primers, as described by the manufacturer. The nucleic acid sequences were aligned with sequences previously recorded in the GenBank using the Clustal W method of the Megalign Software.

Results

From the viable samples (live larvae and pupae) obtained from the collections carried out 3,417 (91%) of the specimens belonged to the species *A. aegypti* (1,412 males; 2,005 females) and 336 (9%) belonged to *A. albopictus* (124 males; 212 females).

A total of 47 *Aedes* females pools (Table 2) were inoculated in cell cultures, and 3 (6.3%) of them (pools 34, 35 and 49) were positive for DENV by IFA (Figure 2). This result was also confirmed by analysis of the agarose gel electrophoresis profile of these pools submitted to RT-PCR (Figure 3).

Pool 34, comprising 35 *A. albopictus* specimens collected in May 2007 in the neighborhood of Messejana, revealed the presence of DENV-3. Pool 35, comprising 50 *A. aegypti* collected in the neighborhood of Joaquim Távora in January 2008, was found to

Table 2. Infestation rates of neighborhoods of Fortaleza and pools of *A. aegypti* and *A. albopictus* females submitted to IFA and RT-PCR.

Neighborhoods	Infestation rates/Month and year of collection	<i>A. aegypti</i> *	<i>A. albopictus</i> *
1. Barra do Ceará	3.28/Mar. 2007–1.48/Mar. 2009	50– Mar. 2007	–
2. Quintino Cunha	6.51/Mar. 2007–4.35/Jan. 2008 3.35/Mar. 2009	50– Mar. 2007 50– Jan. 2008	48– Mar. 2007
3. Antônio Bezerra	7.27/Mar. 2007–5.74/Jan. 2008 2.83/June 2009	50– Mar. 2007	–
4. Pici	3.96/Mar. 2007–2.51/Jan 2008	49– Mar. 2007	–
5. Henrique Jorge	5.88/Mar. 2007–3.64/Jan. 2008 2.54/Mar. 2009	50– Mar. 2009	–
6. Conjunto Ceará I	8.93/Mar. 2007–6.39/Jan. 2008 4.47/Mar. 2009	50– Jan. 2008	–
7. João XXIII	2.94/Mar. 2007–3.36/June 2009	50– June 2009	–
8. Conjunto Ceará II	6.79/Mar. 2007–5.63/Jan. 2008 3.98/Mar. 2009	50– Mar. 2007	–
9. Granja Lisboa	4.61/Mar. 2007–5.71/Jan. 2008	50– Jan. 2008	50– Jan. 2008
10. Siqueira	1.33/June 2007–2.34/Mar. 2009	50– June 2007	–
11. Bom Jardim	3.45/Mar. 2007–5.79/Jan. 2008 2.30/June 2009	49– June 2009	–
12. Granja Portugal	2.95/Mar. 2007–6.76/Jan. 2008 3.40/June 2009	50– Jan. 2008	–
13. Bom Sucesso	5.08/Mar. 2007–3.86/Jan 2008 3.45/June 2009	50– Mar. 2007	–
14. Vila Pery	2.65/Mar. 2007–3.24/Jan. 2008 3.26/Mar. 2009	50– Mar. 2009	–
15. Vila Manoel Sátiro	2.04/Mar. 2007–2.22/Mar. 2009	50– Mar. 2007	–
16. Mondubim	3.12/June 2007–1.76/Mar. 2009	50– June 2007	–
17. Canindezinho	1.88/Mar. 2007–1.46/Mar. 2009	41– Mar. 2007	–
18. José Walter	4.25/Mar. 2007–3.14/Mar. 2009	50– Mar. 2007	–
19. Itaperi	3.24/Mar. 2007–3.51/Jan. 2008 2.84/Mar. 2009	50– Jan. 2008	–
20. Itaoca	1.34/June 2007–2.16/Jan. 2008 1.72/Mar. 2009	50– Jan. 2008	–
21. Montese	5.05/Mar. 2007–3.13/Jan. 2008	50– Mar. 2007	18– Mar. 2007
22. Vila União	1.76/Mar. 2007–3.27/Jan. 2008 2.29/June 2009	50– Jan. 2008	–
23. Rodolfo Teófilo	5.78/Mar. 2007–2.91/Jan. 2008 4.33/Mar. 2009	50– Mar. 2007	–
24. Centro	3.50/Mar. 2007–4.21/Jan. 2008 2.67/Mar. 2009	50– Mar. 2009	–
25. Meireles	1.28/Mar. 2007–5.59/Jan. 2008	50– Jan. 2008	–
26. Aldeota	2.89/Mar. 2007–5.97/Jan. 2008 3.69/Oct. 2008	47– Oct. 2008	–
27. Joaquim Távora	4.78/Mar. 2007–3.34/Jan. 2008	50– Jan. 2008	–
28. Dionísio Torres	4.78/Mar. 2007–5.99/Jan. 2008 2.99/Mar. 2009	47– Mar. 2007	–
29. São João Tauape	3.28/July 2007–2.67/Jan. 2008	24– Jan. 2008	41– July 2007
30. Jardim das Oliveiras	1.98/Mar. 2007–1.89/June 2009	48– Mar. 2007	–
31. Parque Manibura	5.32/Dec. 2007–4.11/Jan. 2008	50– Dec. 2007	–
32. Messejana	2.03/May 2007–3.48/Jan. 2008 2.17/July 2009	50– July 2009	35– May 2007
33. Curió	4.00/Mar. 2007–5.59/Jan. 2008 3.90/June 2009	50– Mar. 2007	–
34. Paupina	2.18/Mar. 2007–1.29/June 2009	50– June 2009	20– June 2009
35. Mucuripe	3.03/Jan. 2008–2.96/Mar. 2009	50– Jan. 2008	–
36. Cais do Porto	2.73/Jan. 2008–1.50/Mar. 2009	50– Jan. 2008	–

Table 2. Cont.

Neighborhoods	Infestation rates/Month and year of collection	<i>A. aegypti</i> *	<i>A. albopictus</i> *
37. Vicente Pinzon	3.12/June 2007–2.92/Mar. 2008	50– June 2007	–
38. Praia do Futuro I	5.45/Dec. 2007–1.20/June 2009	50– Dec. 2007	–
39. Praia do Futuro II	1.58/Mar. 2007–2.23/Jan. 2008	50– Jan. 2008	–
40. Cocó	2.23/Jan. 2008	50– Jan. 2008	–

*Mosquitoes/pool – month and year of collection. In bold, positive pools by both IFA and RT-PCR. The other pools were negative for both tests.
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be infected with DENV-2. Pool 49, comprising 41 *A. albopictus* collected in July 2007 in a park called Parque Adahil Barreto (3° 45' 16" S and 38° 30' 03" W), located in the neighborhood of São João Tauape, revealed the simultaneous presence of DENV-2 and DENV-3.

From the results obtained, was estimated a minimum infection rate (MIR) of 0.5 (1 positive pool ÷ 2,005 mosquitoes tested × 1000) for *A. aegypti*, while MIR for *A. albopictus* was 9.4 (2 positive pools ÷ 212 mosquitoes tested × 1000).

The nucleotide sequencing of samples relating to DENV-3, obtained from pools 34 and 49, resulted in two fragments of 192 bp and 152 bp, respectively. These sequences were registered in GenBank with the access codes HM130699 and JF261696. The sequence obtained for the DENV-2 showed an electropherogram difficult to be analyzed (overlapping peaks), even after repeated sequencing reactions, thus not included in the results.

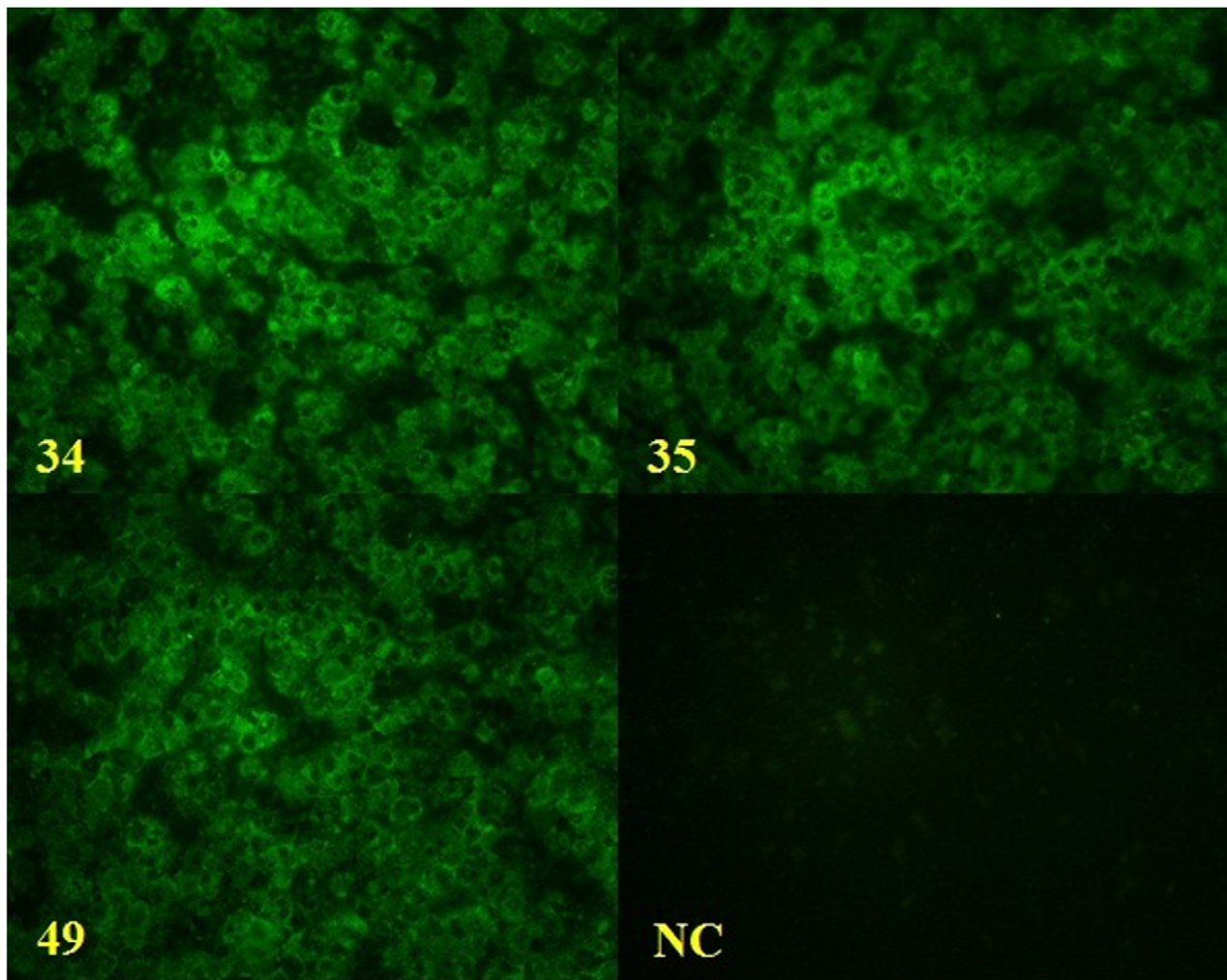


Figure 2. Indirect fluorescent antibody test of *Aedes* mosquitoes pools. Pools 34 (A), 35 (B) and 49 (C) were positive for DENV; NC = negative control.

doi:10.1371/journal.pone.0041386.g002

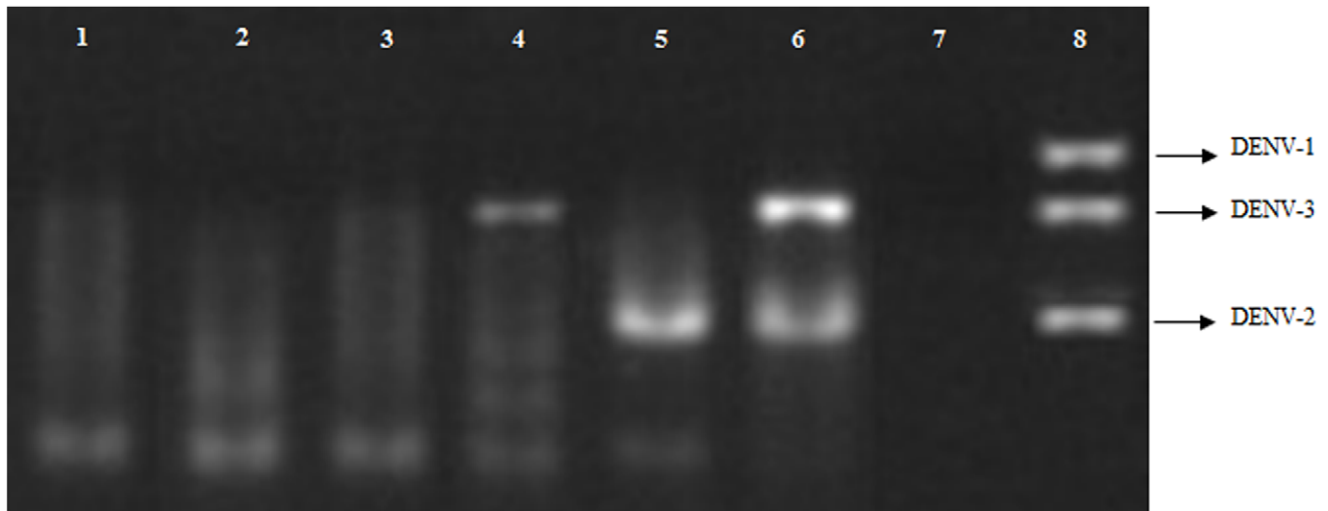


Figure 3. Electrophoresis in agarose gel of the products of RT-PCR. Wells 1, 2 and 3 indicate negative pools for the dengue virus; well 4 reveals genomic fragment of DENV -3, obtained from the pool 34; well 5 reveals genomic fragment of DENV-2 obtained from the pool 35; well 6 reveals simultaneously genomic fragments of DENV-2 and DENV-3, obtained from the pool 49; well 7 contains the negative control, and well 8 contains a mix of DENV-1, DENV-2 and DENV-3 (positive control).
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Discussion

The isolation and the genome fragments detection of DENV-2 and DENV-3 in adult females of *A. aegypti* and *A. albopictus*, deprived of having a blood meal, recorded, for the first time, the occurrence of the vertical transmission of DENV in Ceará State.

These facts support the DENV-1 and DENV-2 isolation from *A. albopictus* larvae collected in 2003 in Belo Horizonte, capital of Minas Gerais State (Southeast region of Brazil) [22]. In the same city, DENV-3 was isolated from *A. aegypti* eggs collected during the period of 2000 to 2004 [23]. Guedes et al. [24] isolated DENV-1, DENV-2 and DENV-3 from adult *A. aegypti* females which emerged from eggs collected in Recife, capital of Pernambuco State (Northeast region of Brazil), in 2005 and 2006. Figueiredo et al. [25], in 1999, verified the occurrence of the vertical transmission of DENV-3 in *A. albopictus* larvae, and of DENV-1 in adult females of *A. aegypti*, all specimens originating from the city of Santos, in São Paulo State (Southeast region of Brazil). The same researchers also isolated DENV-2 from female adults of *A. aegypti*, in 2005, in the city of Foz do Iguaçu, in Paraná State (South region of Brazil).

The first report of the circulation of DENV-2 in Ceará State was in 1994, when 47,221 cases of dengue were recorded (26 dengue hemorrhagic fever) [26]. The circulation of this serotype was maintained for the following eight years when, in 2003, DENV-3 was isolated for the first time [27].

Since the appearance of the DENV-3 serotype in Ceará State, the absence of the concomitant circulation of DENV-2 has been observed. This dominance of DENV-3 in relation to DENV-2 has also been observed in Rio de Janeiro State, during the period of 2000–2001, when DENV-3 was detected in 97.8% of the clinical samples submitted to the study [28]. During this study, virological surveillance of the Ceará State detected the circulation of DENV-2 and DENV-3 in Fortaleza by tests performed with sera from human patients [27].

In Brazil, monitoring of DENV by virus isolation and RT-PCR in *Aedes* spp has been performed sporadically by a few researches [29–31] and has not been incorporated as a routine activity in dengue control programs. RT-PCR is a powerful tool in

virological surveillance of DENV, especially when negative results are obtained by other tests such as virus isolation in cell culture [32]. Based on its high sensitivity, RT-PCR is used in epidemiological studies where large amounts of mosquitoes are collected. Our results corroborate those obtained by Urdaneta et al. [33], in which was observed a high sensitivity level of RT-PCR in *A. aegypti* pool sizes up to 20 *A. aegypti* mosquitoes.

There are different factors that govern the contact between humans and *Aedes* mosquitoes. The exposure rate of humans to the vectors of DENV increases the risk of infection by these viruses [34]. The Adahil Barreto Park has a vast covering of plants in its surrounds, enabling favorable conditions for the appearance and maintenance of *A. albopictus*, as observed by Alencar [unpublished data]. This area shows an intense flux of people, serving as a place for the practicing of sports and other leisure activities by the population, it is an excellent point of contact between humans and specimens of *A. albopictus* which can take advantage of this situation to carry out their blood sucking.

Thus, the detection of DENV in specimens of *A. albopictus* prompts discussion regarding the transmission of DENV to the people who visit the Adahil Barreto Park and its dissemination to other areas, mainly when they return to their homes, where there is a predominance of *A. aegypti* [35]. This potential of *A. albopictus* to act as a bridge for the introduction of this arbovirus in peridomestic environments is a factor which increases the risk of human infection [36].

In Brazil, there have been reports of the isolation of DENV from populations of *A. aegypti* in Distrito Federal (Center-West region of Brazil) [37], in Nova Iguaçu (Rio de Janeiro State – Southeast region of Brazil) [30], and in Manaus (capital of Amazonas State – North region of Brazil) [29,38]. However, these findings did not document the phenomenon of the vertical transmission of DENV in *A. aegypti* occurring in Brazil, since the females submitted to the virus isolation tests had not been deprived of blood feeding. On the other hand, in our study, the detection of DENV in adult females deprived of blood feeding reinforces the vertical (transovarian) transmission of DENV in *Aedes* mosquitoes as an important mechanism in the maintenance of this virus in nature.

Table 3. Records of the occurrence of vertical transmission of DENV in *A. aegypti* and *A. albopictus*.

Stage of insect development	Species	DENV isolate/Detected by	Country/Reference
^a Adult (♂)	<i>A. aegypti</i>	2/head squash-direct fluorescence antibody	Myanmar (14)
^b Adult *	<i>A. aegypti</i>	4/indirect fluorescence antibody and complement fixation	Trinidad and Tobago (49)
^a Adult *	<i>A. aegypti</i>	3/indirect fluorescence antibody	India (50)
^c Adult (♂)	<i>A. albopictus</i>	2, 3/indirect fluorescence antibody and RT-PCR	Mexico (51)
^c Adult (♂ and ♀)	<i>A. aegypti</i> and <i>A. albopictus</i>	1/RT-PCR	Singapore (52)
^a Adult (♂ and ♀)	<i>A. aegypti</i>	2, 3/ELISA and <i>Toxorhynchites splendens</i> inoculation-indirect immunofluorescence	India (53)
Larvae	<i>A. aegypti</i> and <i>A. albopictus</i>	♦/peroxidase anti peroxidase staining	Malaysia (54)
^c Adult (♂)	<i>A. aegypti</i> and <i>A. albopictus</i>	1, 2, 3, 4/RT-PCR	Singapore (55)
^a Adult (♀)	<i>A. aegypti</i>	2, 3, 4/RT-PCR	Mexico (56)
Larvae and ^a adult (♂ and ♀)	<i>A. albopictus</i>	2/ELISA and indirect fluorescence antibody	India (57)
Larvae	<i>A. aegypti</i> and <i>A. albopictus</i>	1, 3/Indirect fluorescence antibody and RT-PCR	Malaysia (58)
Larvae and ^a adult (♂ and ♀)	<i>A. aegypti</i>	2/RT-PCR	Indonesia (59)
^a Adult *	<i>A. aegypti</i> and <i>A. albopictus</i>	♦/Indirect fluorescence antibody	India (60)
^c Adult (♂)	<i>A. aegypti</i>	2, 3/ELISA and <i>Toxorhynchites splendens</i> inoculation-indirect immunofluorescence	India (61)
^a Adult *	<i>A. aegypti</i>	♦/head squash- direct fluorescence antibody	Indonesia (62)
^a Adult (♂ and ♀)	<i>A. aegypti</i> and <i>A. albopictus</i>	♦/ELISA	India (63)
^a Adult *	<i>A. aegypti</i>	1,2, 3, 4/RT-PCR	Thailand (64)

^aAdults that emerged from larvae collected in the field;

^bAdults that emerged from eggs collected in the field;

^cAdults collected in the field;

(*)No information available on the sex of the mosquitoes tested;

(♦)Serotypes not specified.

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There have been several reports on the vectorial competence and capacity of different populations of *A. aegypti* and *A. albopictus* in relation to DENV. In many of these studies the mosquitoes were infected artificially with different strains and serotypes of DENV and, in parallel, attempts were made to observe the occurrence of the vertical transmission of this virus to progeny [15,39–48]. On the other hand, there are some references regarding to the natural occurrence of the vertical transmission of DENV in the species *A. aegypti* and *A. albopictus*. In these studies, it was possible to identify DENV in adult mosquitoes emerging from both larvae and eggs collected in the field, deprived of a blood feeding, and adult collected directly in the field (Table 3) [14,49–64].

The epidemiological relevance of the role of vector mosquitoes in the transmission of this arbovirus within a certain period can be estimated through the minimum infection rate (MIR), which may serve as a tool for predicting epidemics [65]. The MIR values observed in our study points to its useful for the prediction of epidemic episodes of dengue, since in 2008 the largest epidemic of the disease in Fortaleza was recorded, with the circulation of DENV-2 and DENV-3, and 34,109 confirmed cases [4]. This is a notable result since *A. albopictus* is not considered as a vector of the dengue virus in Brazil. Surveillance of DENV vectors allows timely implementation of emergency mosquito control measures such as insecticidal fogging of adults and destruction of breeding sites to inhibit an impending outbreak from spreading [52].

Considering these results and the adaptive potential of both species to colonize a wide variety of types of breeding sites in the

urban environment of Fortaleza, as demonstrated by Martins et al. [35], it is necessary to expand the strategies directed toward combating these Culicidae in the Dengue Control Programs in Fortaleza, especially in relation to *A. albopictus*. Although there are no confirmed cases in the literature of the transmission of DENV by populations of *A. albopictus* in episodes of epidemics in Brazil, this possibility cannot be discarded. Furthermore, the occurrence of the vertical transmission of DENV-2 and DENV-3 in *A. aegypti* and *A. albopictus* in Fortaleza opens discussion regarding the role performed by this viral transmission mechanism in the maintenance of DENV in nature during interepidemic periods in Brazil.

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Author Contributions

Conceived and designed the experiments: VEPM CHA MTK FMCA. Performed the experiments: VEPM CHA MTK FMCA RFD. Analyzed the data: VEPM CHA MTK FMCA MIFG SGDS RFD. Contributed reagents/materials/analysis tools: MIFG SGDS RFD. Wrote the paper: VEPM CHA FMCA MIFG. Identification of Aedes adults mosquitoes: VEPM CHA. Nucleotide sequencing: MTK VEPM. Description of the methodology for cell culture: FMCA SGDS VEPM.

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