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RESEARCH ARTICLE

Culex quinquefasciatus from Rio de Janeiro Is Not Competent to Transmit the Local Zika Virus

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Abstract

Background
The Americas have suffered a dramatic epidemic of Zika since May in 2015, when Zika virus (ZIKV) was first detected in Brazil. Mosquitoes belonging to subgenus Stegomyia of Aedes, particularly Aedes aegypti, are considered the primary vectors of ZIKV. However, the rapid spread of the virus across the continent raised several concerns about the transmission dynamics, especially about potential mosquito vectors. The purpose of this work was to assess the vector competence of the house mosquito Culex quinquefasciatus from an epidemic Zika area, Rio de Janeiro, Brazil, for local circulating ZIKV isolates.

Methodology/Principal Findings
Culex quinquefasciatus and Ae. aegypti (positive control of ZIKV infection) from Rio de Janeiro were orally exposed to two ZIKV strains isolated from human cases from Rio de Janeiro (Rio-U1 and Rio-S1). Fully engorged mosquitoes were held in incubators at 26 ± 1°C, 12 h:12 h light:dark cycle and 70 ± 10% humidity. For each combination mosquito population—ZIKV strain, 30 specimens were examined for infection, dissemination and transmission rates, at 7, 14 and 21 days after virus exposure by analyzing body (thorax plus abdomen), head and saliva respectively. Infection rates were minimal to completely absent in all Cx. quinquefasciatus-virus combinations and were significantly high for Ae. aegypti. Moreover, dissemination and transmission were not detected in any Cx. quinquefasciatus mosquitoes whatever the incubation period and the ZIKV isolate. In contrast, Ae. aegypti ensured high viral dissemination and moderate to very high transmission.
Conclusions/Significance

The southern house mosquito *Cx. quinquefasciatus* from Rio de Janeiro was not competent to transmit local strains of ZIKV. Thus, there is no experimental evidence that *Cx. quinquefasciatus* likely plays a role in the ZIKV transmission. Consequently, at least in Rio, mosquito control to reduce ZIKV transmission should remain focused on *Ae. aegypti*.

Author Summary

The pandemic Zika epidemic has affected nearly all American countries. The etiological agent is a mosquito-borne-virus originated from Africa that spread to Asia and more recently, to the Pacific region and the Americas. We experimentally demonstrated that the common house nightly biting mosquito *Culex quinquefasciatus* from Rio de Janeiro was not susceptible to locally circulating Zika virus (ZIKV) strains. Dissemination was not observed in *Cx. quinquefasciatus* regardless of the ZIKV isolate used and the incubation period after the ingestion of an infected blood meal. No infectious ZIKV particle was detected in the saliva of the four *Cx. quinquefasciatus* populations examined until 3 weeks after virus exposure. In contrast, we confirmed that local *Aedes aegypti* mosquitoes can be infected, disseminate ZIKV at significantly high rates, and assured moderate to very high viral transmission after day 14 of virus exposure. We concluded that *Cx. quinquefasciatus* is not competent to transmit local ZIKV. Our results support that mosquito control should focus on *Ae. aegypti* to reduce Zika transmission.

Introduction

A Zika virus (ZIKV) epidemic has rapidly spread throughout tropical and subtropical zones of the American continent since early 2015 [1]. Brazil was likely the starting point of the Zika pandemic in the Americas [2, 3]. The Zika virus pandemic has spread to North America too. By July 2016, 45 American countries or territories have already reported active ZIKV transmission (http://www.cdc.gov/zika/geo/active-countries.html).

ZIKV is a positive-sense, single-stranded RNA mosquito-borne-virus of 10,807 nucleotides belonging to family *Flaviviridae*, genus *Flavivirus*. It is composed of three major lineages: East African, West African, and Asian [4]. ZIKV was first isolated from a sentinel rhesus monkey in the Zika forest in Uganda in 1947 [5]. The second ZIKV isolations were obtained from 20 pools of the forest canopy feeder mosquito *Aedes (Stegomyia) africanus* captured in the same area [6].

Almost 70 years have passed and little is known about natural ZIKV vectors. *Aedes* mosquitoes are considered the primary vectors of ZIKV in Africa with reported viral isolations from several species, especially from *Ae. africanus* [1, 7–10]. ZIKV was also isolated from several other mosquito species belonging to genus *Aedes* (subgenera *Stegomyia* and *Diceromyia*), *Mansonia* and *Culex*, and horse flies from the wild in Uganda [8]. More recently, natural infections screened by molecular methods in sylvatic African mosquitoes were again predominantly found in *Aedes* belonging to subgenus *Stegomyia*, but also in other species of *Aedes, Mansonia, Culex*, *Anopheles* [9, 10]. Nevertheless, ZIKV transmission in the wild has remained poorly understood. Only two sylvatic species (*Ae. vittatus* and *Ae. luteocephalus*) proved to be able to transmit ZIKV in laboratory assays [11].
The domestic mosquito *Ae. (Stegomyia) aegypti* was early shown to be competent to experimentally transmit ZIKV [12]. Due to its high anthropophilic and domestic behaviors and virus detection in field caught specimens [13, 14], this mosquito has been incriminated as the urban and periurban vector in Africa and Asia [1,15].

ZIKV has only recently emerged outside of its natural distribution in Africa and Asia, and has caused a series of epidemics in urban and periurban sites on Pacific islands [16–20] before reaching the Americas, probably in 2013 [21]. The spreading virus belonged to the Asian genotype [21]. Despite multiple efforts, mosquito vectors involved in the ZIKV outbreaks across the Pacific Ocean in 2007–2015 were not identified. *Ae. aegypti* and other local members of subgenus *Stegomyia* (*Ae. hensilli* and *Ae. polynesiensis*) were thought to be potential vectors [16, 22, 23]. *Ae. (Stegomyia) albopictus* was found naturally infected with ZIKV in urban sites in Gabon in 2007 [24] and Mexico (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=34243&lang=en). Additionally, *Ae. aegypti* from Singapore were competent to transmit the African ZIKV genotype in the laboratory [25]. Thereafter, *Ae. albopictus* has been considered a potential vector of ZIKV throughout its geographical range, concomitantly or not with *Ae. aegypti* [1, 24, 26, 27].

With the arrival of the ZIKV Asian genotype in the Americas, the global number of suspected and confirmed ZIKV cases reached levels never seen previously [28, 29]. Besides, the rapid geographical spread, the increased incidence of severe congenital troubles, such as microcephaly, and Guillian-Barré syndrome associated with ZIKV in Brazil led the World Health Organization to declare the ZIKV epidemic a Public Health Emergency of International Concern [1, 30]. ZIKV proved to have a high potential for geographic expansion in regions wherever *Ae. aegypti* mosquitoes are present, concomitantly with Dengue viruses 1–4 and Chikungunya virus prone areas of transmission, as it has occurred in Brazil and other American tropical and subtropical countries [29, http://www.cdc.gov/zika/geo/active-countries.html]. American *Ae. aegypti* and *Ae. albopictus* populations showed to be competent to transmit the ZIKV belonging to the circulating genotype, but displayed heterogeneous infection, dissemination and transmission rates in laboratory assays [26]. However, *Ae. aegypti* and *Ae. albopictus* populations from Brazil and USA exhibited low transmission efficiency to ZIKV [26], which appeared inconsistent with the rapid Zika spread throughout the Americas. Two main hypotheses might explain this scenario: (1) The large number of humans susceptible to ZIKV combined with high densities of anthropophilic *Aedes* mosquitoes compensate their relatively low vector competence to ZIKV [26]. (2) Although the recent ZIKV pandemic has occurred only in *Stegomyia*-infested zones and *Ae. aegypti* has been suggested to be the main vector, other anthropophilic, domestic and usually abundant mosquitoes such as *Culex* species could contribute to ZIKV transmission [1, 31]. For example, *Culex* species belonging to the Pipsiens Assemblage [32], such as *Cx. quinquefasciatus*, were likely candidate due their high human-biting frequency and distribution in urban epidemic centers (http://www.reuters.com/article/us-health-zika-brazil-idUSKCN0W52AW). There is no information whether *Cx. quinquefasciatus* can transmit the virus or the potential role of this mosquito in the ZIKV transmission in nature. We herein comparatively assess the vector competence of *Cx. quinquefasciatus* and *Ae. aegypti* populations from Rio de Janeiro for two local ZIKV isolates.

**Materials and Methods**

**Mosquitoes**

*Cx. quinquefasciatus* populations tested in this study were collected from four districts of Rio de Janeiro: Manguinhos (MAN, 22°52'20"S 43°14'46"W), Triagem (TRI, 22°53'56"S 43°14'44"W) Copacabana (COP, 22°58'8.3"S 43°11'21"W) and Jacarepaguá (JAC, 22°57'42"S 43°
24°11'W). For comparison, we used two populations of Ae. aegypti from Rio de Janeiro, Brazil: Urca (URC, 22°56′45″S 43°09′43″W) and Paquetá (PAQ, 22°45′44″S 43°06′26″). The mosquitoes were concurrently collected as larvae or eggs using ovitraps from January to March 2016 to initiate laboratory colonies. Each colony was started with at least 200 field-collected individuals from more than five distinct collecting sites and traps. Field collected larvae and eggs were hatched and reared in insectaries (26 ± 1°C; 70 ± 10% RH; 12 h:12 h light:dark cycle). Larvae were reared in pans (~100 larvae/pan measuring 30 x 21 x 6 cm) containing 1 liter of dechlorinated tap water supplemented with yeast tablets. Adults were kept under the same insectary controlled conditions described above, and supplied with a 10% sucrose solution. All experimental oral infections were performed with mosquitoes of the F1 generation, except for TRI (laboratory colony) and PAQ (F2).

Viral strains
Mosquitoes were challenged with two ZIKV strains of the Asian genotype, named Rio-U1 and Rio-S1, respectively isolated from urine and saliva of two patients in January 2016, living in distinct districts in Rio de Janeiro [33]. The viral samples were isolated, kept anonymized and provided by Bonaldo et al. [33], whose the institutional review board at Fundação Oswaldo Cruz has previously approved their study protocol. Viral stocks were obtained after two passages of the isolates onto Vero cells maintained with Earle’s 199 medium supplemented with 5% fetal bovine serum (FBS), under an atmosphere containing 5% CO₂, and incubated at 37°C. Viral titer in supernatants were estimated by plaque-forming unit (PFU) assays of serial dilutions on Vero cells maintained at 37°C for 7 days and expressed in PFU/mL. Samples were kept at -80°C until use. The comparison of genomic sequences of ZIKV strains Rio-U1 (KU926309) with Rio-S1 (KU92630) yielded 99.6% identity, displaying six amino acid variations in the viral proteins. Phylogenetic analysis showed 99.7% identity of Rio-U1 and Rio-S1 strains with ZIKV isolates from Guatemala and other Brazil regions, including a Zika-associated microcephaly case. They all cluster (bootstrap score = 97%) within the Asian genotype and share a common ancestor with the ZIKV strain that circulated in French Polynesia in November 2013 [33].

Mosquito experimental assays
Five to seven day-old females were isolated in feeding boxes and starved for 24 h and 48 h for Aedes and Culex mosquitoes, respectively. All mosquitoes were exposed to the infectious blood-meal containing a final viral titer of 10⁶ PFU/mL which consists of a mixture of two parts of washed rabbit erythrocytes and one part of the viral suspension added with a phagostimulant (0.5 mM ATP). Females were fed through a pig-gut membrane covering the base of glass feeders containing the infectious blood-meal maintained at 37°C. Mosquito feeding was limited to 60 min. Only fully engorged females were incubated at 26°C constant temperature, 70 ± 10% RH and 12 h:12 h light:dark cycle, with daily access to 10% sucrose solution. When available, samples of 30 mosquitoes of each population were examined at 7, 14 and 21 days after virus exposure, hereinafter abbreviated as “dpi”.

Mosquitoes were individually processed as follows: abdomen and thorax (herein after referred to as body) were examined to estimate viral infection rate, head for dissemination and saliva for transmission. Each female was handled at a time, by using disposable and disinfected supplies to avoid contamination between individuals and between tissues of the same mosquito as previously described [34]. For the determination of viral infection and dissemination rates, each mosquito body and head were respectively ground in 500 μL and 250 μL of medium supplemented with 4% FBS, and centrifuged at 10,000 x g for 5 min at +4°C before titration. Body and head homogenates were serially diluted and inoculated onto monolayers of Vero cells in
96-well plates. After 1 h incubation of homogenates at 37° C, 150 μL of 2.4% CMC (carboxymethyl cellulose) in Earle’s 199 medium was added per well. After 7 days incubation at 37° C, cells were fixed with 10% formaldehyde, washed, and stained with 0.4% crystal violet. Presence of viral particles was assessed by detection of viral plaques. Additionally, body and head homogenates were individually submitted to specific ZIKV RNA detection and quantification through RT-qPCR, using the SuperScript III Platinum one-step RT-qPCR (Invitrogen) in QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). For each reaction, we used 600 nM forward primer (5’-CTTGGAGTGCTTGTGATT-3’, genome position 3451–3468), 600 nM reverse primer (5’-CTCCTCCAGTGTTCATTT-3’, genome position 3637–3620) and 800 nM probe (5’FAM- AGAAGAGAATGACCACAAAGATCA-3’TAMRA, genome position 3494–3517). The sequences of this primer set were provided by Isabelle Lepark-Goffart (French National Reference Centre for Arboviruses, IRBA, Marseille, France). The reverse transcription was performed at 45° C for 15 min. The qPCR conditions were 95° C for 2 minutes, followed by 40 amplification cycles of 95° C for 15 sec, 58° C for 5 sec and 60° C for 30 sec. For each run, numbers of ZIKV RNA copies were calculated by absolute quantitation using a standard curve, whose construction details are described elsewhere [33].

In order to assess the transmission rate (TR) and transmission efficiency (TE), mosquito saliva was collected in individual pipette tips containing 5 μL FBS and processed by PFU assays, as previously described [26]. Accordingly, mosquito saliva was inoculated onto Vero Cell monolayer in 6-well plates incubated 7 days at 37° C, under 3 mL with 2.4% CMC in Earle’s 199 medium overlay, and stained as described above. Viral titers of saliva were expressed as PFU/saliva.

Infection rate (IR) refers to the proportion of mosquitoes with infected body (abdomen and thorax) among tested mosquitoes. Disseminated infection rate (DIR) corresponds to the proportion of mosquitoes with infected head among tested mosquitoes (i.e.; abdomen/thorax positive). Transmission efficiency (TE) represents the proportion of mosquitoes with infectious saliva among the initial number of mosquitoes tested. Transmission rate (TR) represents the proportion of mosquitoes with infectious saliva among mosquitoes with disseminated infection.

Statistical analysis
To compare the viral load, the Wilcoxon signed rank test was adopted to analyze pairwise comparison at 7, 14 and 21 dpi for each mosquito population and tested virus strain. Significant difference was established when p-values were lower than 0.05. Data analyses were conducted with PRISM 5.0 software (GraphPad Software, San Diego-CA, USA, 2007).

Ethics statements
This study was approved by the Institutional Ethics Committee on Animal Use (CEUA-IOC license LW-34/14) at the Instituto Oswaldo Cruz. No specific permits were required for performing mosquito collection in the districts in Rio de Janeiro.

Results
*Cx. quinquefasciatus* infrequently become infected with ZIKV
We comparatively evaluated the susceptibility to infection of *Cx. quinquefasciatus* and *Ae. aegypti* from Rio de Janeiro to two ZIKV strains locally isolated. Infection rates (IR) were negligible to null in *Cx. quinquefasciatus*, whereas they remained very high for *Ae. aegypti* (Fig 1A). With few exceptions, the IRs were of 100% in the two tested *Ae. aegypti* populations (URC and
PAQ) at 14 and 21 dpi, for both virus isolates. In addition, when examining Ae. aegypti from URC, 80% have already been infected by 7 dpi (Fig 1A). In contrast, none of the four Cx. quinquefasciatus populations was likely to become infected except for 1 of 30 TRI Cx. quinquefasciatus challenged with ZIKV Rio-U1, at 14 dpi (viral load: 1,814 RNA copies/ml; 7.0 PFU/ml) (Fig 1A). ZIKV RNA copies (1,453 RNA copies/ml) were detected in 1 of 16 MAN Cx. quinquefasciatus at 14 dpi challenged with the same ZIKV strain. However, infective viral particles were not detected in the homogenate of this specimen in repeated PFU assays. Viral load estimated in bodies of Ae. aegypti tended to increase with incubation time (Fig 2), and the lowest values being detected at 7 dpi (median: 1.1 x 10^6 RNA copies/ml, mean ± SE: 2.3 x 10^5 ± 2.4 x 10^6 RNA copies/ml) and the highest at 21 dpi (median: 1.5 x 10^9 RNA copies/ml, mean ± SE:
1.3 x 10^9 ± 8.3 x 10^8 RNA copies/ml). Accordingly, viral load was significantly higher at 21 dpi than at 7 (p = 0.0098) and 14 dpi (p = 0.009). Viral loads at 14 dpi in bodies of *Ae. aegypti* from PAQ [IR: 100%, Fig 1; viral load: 1.6 x 10^8 RNA copies/mL (median); 2.6 x 10^8 ± 2.8 x 10^8 RNA copies/mL (mean ± SE), Fig 2] were significantly higher than for URC [IR: 90.9%, Fig 1, viral load: 2.1 x 10^7 RNA copies/mL (median); 2.6 x 10^8 ± 4.3 x 10^8 RNA copies/mL (mean ± SE), Fig 2] when challenged with the same ZIKV isolate (Rio-U1).

The circulating ZIKV can promptly disseminate and efficiently be transmitted by *Ae. aegypti*, but not by *Cx. quinquefasciatus* from Rio

*Cx. quinquefasciatus* did not showed viral dissemination regardless of the incubation period whereas dissemination infection rates (DIR) were consistently high (~85–97%) in *Ae. aegypti* at 14 and 21 dpi irrespective the ZIKV strain (Fig 1B). Accordingly, transmission determined by detecting infective viral particles in mosquito saliva was not observed in any pair of *Cx. quinquefasciatus* population-ZIKV strain regardless the time point of examination (Fig 1C). In contrast, significantly high transmission rates (TR: 71.6–96.5%) and transmission efficiency (TE: 60.6–93.3%) were observed in local *Ae. aegypti* (PAC and URC) at 14 dpi (Fig 1C and 1D).

At 14 dpi, viral load in the head of *Ae. aegypti* from URC infected with ZIKV Rio-S1 (Fig 2) were significantly higher (median: 1.2 x 10^7 RNA copies/mL; mean ± SE: 1.4 x 10^7 ± 9.5 x 10^6 RNA copies/mL) compared to ZIKV Rio U1 (median: 3.6 x 10^6 RNA copies/mL mean ± SE: 6.3 x 10^6 ± 7.8 x 10^6 RNA copies/mL, Fig 2) (p = 0.0003). When challenged with the same ZIKV isolate (Rio-U1), viral load in heads at 14 dpi was significantly higher in *Ae. aegypti* from PAQ (median: 1.8 x 10^7 RNA copies/mL, mean ± SE: 3.7 x 10^7 ± 5.0 x 10^6 RNA copies/mL, Fig 2) than URC (p = 0.0018). As expected, DIR was lower (DIR = 40%) in *Ae. aegypti* (URC) at 7 dpi, and no transmission was observed at this time point (Fig 1B–1D). TRs and TEs at 14 dpi
were higher for PAQ compared to URC *Ae. aegypti* challenged with the same ZIKV isolate (Rio-U1) (Fig 1C and 1D), although viral load did not differ (p = 0.4203) between mosquito populations (Fig 3). Also, comparisons of viral loads in saliva of URC *Ae. aegypti* challenged with different ZIKV isolates did not show any difference (40.3 ± 64.5 PFU Rio-S1/saliva versus 34.2 ± 69.0 PFU Rio-U1/saliva; p = 0.3388) (Fig 3). No significant difference was apparent (p = 0.2212) in viral load in saliva between 14 and 21 dpi (Fig 3).

**Discussion**

The Zika epidemics has affected nearly all American countries with ca. 445,000 cumulative suspected cases, with 91,962 confirmed infections and 9 deaths due to ZIKV as of August 5, 2016 (http://ais.paho.org/phil/viz/ed_zika_cases.asp). South American countries had nearly 74% of the continental Zika suspected cases, with ca. 5% (165,932 suspected cases) from Brazil. The incidence rate in Brazil is 81.2/100,000 inhabitants Zika suspected cases, with 1,749 cases of microcephaly associated to ZIKV infection diagnosed by clinical, epidemiological and/or laboratory criteria as of May 2016 (http://www.paho.org/hq/index.php?option=com_content&view=article&id=11599&Itemid=41691). Rio de Janeiro is one of the highest risk areas in Brazil, with an incidence of 278.1/100,000 suspected Zika cases as of July 2016 (http://portalsaude.saude.gov.br/images/pdf/2016/julho/15/2016-boletim-epi-n28-dengue-chik-zika-se23.pdf).

To face such a severe health crisis, efficient and effective mosquito control strategies are essential. However, it depends on the definition of primary and/or potential local mosquito vectors. Other ZIKV transmission mechanisms besides *Ae. aegypti* have been observed. For instance, sexual ZIKV transmission between humans has been observed [35]. Natural ZIKV infections detected in several mosquito genera and even in horse flies would suggest that ZIKV could potentially infect a large range of mosquito species and even other hematophagous flies [31, 33, 36]. However, there is no evidence regarding the role of other mosquitoes or flies besides *Aedes* (*Stegomyia*) species in the ZIKV transmission in nature in the Americas. Indeed, there are no data whether other anthropophilic and domestic mosquitoes besides *Ae. albopictus*, and notably *Ae. aegypti* can transmit ZIKV.

In this work, we demonstrate for the first time, under laboratory conditions, that *Cx. quinquefasciatus* are not competent to transmit two ZIKV strains circulating in Brazil. Four tested populations were minimally infected with ZIKV and were unable to transmit this virus. In contrast, two *Ae. aegypti* populations were highly susceptible to ZIKV infection and dissemination, and competent to transmit the same virus strains. This is consistent with *Ae. aegypti* being more likely to sustain the current ZIKV outbreak in Rio de Janeiro and probably in other tropical American zones.

The Zika control program in Brazil, as well as in all epidemic American countries, consists essentially in intensifying and reinforcing the current strategies to control dengue for decades, which focuses in reducing *Ae. aegypti* density and longevity through eliminating or treating potential larval habitats and insecticide spraying (http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf). However, the traditional vector control strategies have usually failed to efficiently reduce dengue transmission and spread, even when properly adopted [38]. Several reasons have been identified to explain these failures, among which are insufficient community engagement and management and high insecticide resistance in the target species, the mosquito *Ae. aegypti* [39–41]. Intensifying *Ae. aegypti* control activities has also been unsuccessful in stemming the rapid spread of ZIKV [1]. Therefore, new technologies are urgently needed to adequately and better mitigate ZIKV transmission, likely requiring combinations of several approaches. For instance, it has been recently demonstrated that *Wolbachia*...
infected *Ae. aegypti* from Brazil blocks ZIKV transmission [42]. In addition, local control programs should design specific control strategies against the potential vector *Ae. albopictus*, since it has been shown to transmit ZIKV in laboratory [25, 26, 37], with ZIKV detections in field-collected specimens [24, http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=34243&lang=en].

The first determination of vector competence to ZIKV in American *Ae. aegypti* populations was conducted with a viral isolate from New Caledonia, as at the time of that evaluation, no local ZIKV strain were available. Nonetheless, the sequence of NS5 gene of ZIKV from New Caledonia displayed 99.4% identity with ZIKV from Brazil [26]. One Brazilian *Ae. aegypti* population, from Tubiacanga, Rio de Janeiro were challenged with the ZIKV New Caledonia. High susceptibility to infection and moderate dissemination rate, but with low transmission were found, suggesting unexpectedly low competence of local *Ae. aegypti* for ZIKV [26]. Our newly data with two *Ae. aegypti* populations from Rio de Janeiro (URC and PAC) orally challenged with two locally circulating ZIKV isolates (Rio-U1 and Rio-S1) revealed very high dissemination and moderate to high transmission. Similar results were found when testing the URC mosquito population with two ZIKV strains isolated in 2015 from other Brazilian cities [42].

Fig 3. ZIKV load in saliva of *Ae. aegypti* from Rio de Janeiro, Brazil, 14 and 21 days after challenge with two locally circulating ZIKV isolates (Rio-U1 and Rio-S1) provided at a titer of $10^6$ PFU/mL. Virus was detected plaque forming unit (PFU) assays on Vero cells.

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differences in vector competence may be explained by the concept that the outcome of transmission depends on the specific pairing of vector and virus genotypes [43]. Similar to other ZIKV strains isolated during the epidemic in Brazil, sequences of virus strains used in the present study clustered with Asian clade, including sequences from New World, Malaysia, Micronesia and Pacific. Thus, the New Caledonian [26] and Brazilian strains are genetically nearly identical. Phylogenetic and molecular clock analyses are consistent with a single introduction of ZIKV from the Pacific area into the Americas, probably more than 12 months before the detection of ZIKV in Brazil [21]. It is possible that some genome evolution not yet identified has rapidly shaped ZIKV to New World *Ae. aegypti* populations, highlighting the genetic specificity and potential for local adaptation between arboviruses and mosquito vectors previously described for dengue [44].

To evaluate the potential role of a mosquito species to transmit an arbovirus like ZIKV requires examination of multiple components governing vectorial capacity, of which vector competence is simply one. Ecological, epidemiological, environmental and climatic factors influence both vector competence and vectorial capacity. Thus, distinct geographical populations of a mosquito species can greatly diverge in their vector competence when exposed to different virus strains, since the outcome of infection depends on the specific combination of mosquito and virus genotypes [45, 46]. Thus, our demonstration that *Cx. quinquefasciatus* from Rio are not able to transmit ZIKV does not completely rule out the possibility that domestic *Culex* mosquitoes from other origins may exhibit different vector competence.

Nevertheless, to now at least, there is no evidence that the southern house mosquito *Cx. quinquefasciatus* is a potential ZIKV vector. Our study with four *Cx. quinquefasciatus* populations from Rio challenged with two recently isolated virus strains from the same location where mosquitoes were collected showed that this species is not competent to transmit ZIKV. Similar result was obtained when the closely related species *Cx. pipiens* from USA was challenged with a ZIKV isolated from Puerto Rico [47]. Moreover, besides being incompetent to transmit ZIKV in the laboratory, neither *Cx. quinquefasciatus* nor any other species of the Pipiens Assemblage has been found naturally infected in the American ZIKV transmission area [48, 49] or during the 2007 Zika outbreaks in the South Pacific island of Yap (Micronesia) [1, 16] and in Gabon [24] where thousands of *Cx. quinquefasciatus* have been screened.

Therefore, there is no reason to think that mosquito control efforts against *Cx. quinquefasciatus* to reduce Zika transmission, at least in Rio de Janeiro, Brazil. Mosquito measures to mitigate ZIKV transmission should remain focused on *Ae. aegypti*.

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### Author Contributions

Conceived and designed the experiments: RLdO PB ABF.

Performed the experiments: RLdO RSF SSC AFdB KABdS RMdM MGdC LMSR.

Analyzed the data: RLdO RSF MCB.

Contributed reagents/materials/analysis tools: RLdO MCB.
Wrote the paper: RLdO ABF PB MCB.

References

1. Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev. 2016; 29(3):487–524. doi: 10.1128/CMR.00072-15 PMID: 27029595
2. Brasil P, Calvet GA, Siqueira AM, Wakimoto M, de Sequeira PC, Nobre A, et al. Zika Virus Outbreak in Rio de Janeiro, Brazil: Clinical Characterization, Epidemiological and Virological Aspects. PLoS Negl Trop Dis. 2016 Apr 12; 10(4):e0004836. doi: 10.1371/journal.pntd.0004836 PMID: 27070912
3. Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015; 110(4):569–72. doi: 10.1590/0074-02760150192 PMID: 26061233
4. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis. 2014; 8(1):e2636. doi: 10.1371/journal.pntd.0002636 PMID: 24421913
5. Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952; 46(5):521–34. PMID:12995441
6. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952; 46:509–20. PMID:12995440
7. Weinbren MP, Williams MC. 1958. Zika virus: further isolations in the Zika area, and some studies on the strains isolated. Trans R Soc Trop Med Hyg. 1958; 52(3):263–8. PMID:13556872
8. Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. Twelve isolations of Zika virus from Aedes (Stegomyia) africanus (Theobald) taken in and above a Uganda forest. Bull World Health Organ. 1964; 31:57–69. PMID: 14230895
9. Diallo D, Sall AA, Diagne CT, Faye O, Ba Y, et al. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One. 2014; 9(10):e10944.
10. Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J. 2013; 10:311. doi: 10.1186/1743-422X-10-311 PMID: 24148652
11. Diagne CT, Diallo D, Faye O, Ba Y, Faye O, Gaye A, et al. Potential of selected Senegalese Aedes spp. mosquitoes (Diptera: Culicidae) to transmit Zika virus. BMC Infect Dis. 2015; 15:492. doi: 10.1186/s12879-015-1231-2 PMID: 26527535
12. Boorman JP, Porterfield JS. A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. Trans R Soc Trop Med Hyg. 1956; 50(3):238–42. PMID:13337908
13. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg. 1969; 18(3):411–5. PMID: 4976739
14. Akoua-Koffi C, Diarrassouba S, Benie VB, Ngbichi JM, Bozoua T, Bosson A, et al. [Investigation surrounding a fatal case of yellow fever in Cote d'Ivoire in 1999]. Bull Soc Pathol Exot. 2001; 94(3):227–30. PMID: 11681215
15. Olson JG, Ksiazek TG, Suhandiman Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. Trans R Soc Trop Med Hyg. 1981; 75(3):389–93. PMID: 6275577
16. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009; 360(24):2536–43. doi: 10.1056/NEJMoa0805715 PMID: 19516034
17. Caio-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French polynesia, South pacific, 2013. Emerg Infect Dis. 2014; 20(6):1085–8. doi: 10.3201/eid2006.140138 PMID: 24856001
18. Dupont-Rouzyrol M, O’Connor O, Calvez E, Daures M, John M, Grangeon JP, et al. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. Emerg Infect Dis. 2015; 21(2):381–2. doi: 10.3201/eid2102.140153 PMID: 25625687
19. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol. 2015.
20. Musso D, Nilles EJ, Caio-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect. 2014; 20(10):O595–6. doi: 10.1111/1469-0691.12707 PMID: 24909208
21. Faria NR, Azevedo R do S, Kraemer MU, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science 2016; 352: 345–349. doi: 10.1126/science.aaf5036 PMID: 27013429
22. Ledermann JP, Guillamot L, Yug L, Saweyog SC, Tided M, Machieng P, et al. Aedes hensilli as a potential vector of Chikungunya and Zika viruses. PLoS Negl Trop Dis. 2014; 9:8(10):e3188. doi: 10.1371/journal.pntd.0003188 PMID: 25299181

23. Iooe S, Mallet HP, Leparc Goiffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Med Mal Infect. 2014 Jul; 44(7):302–7. doi: 10.1016/j.medmal.2014.04.008 PMID: 25001879

24. Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, et al. Zika virus in Gabon (Central Africa)-2007: a new threat from Aedes albopictus? PLoS Negl Trop Dis. 2014; 8(2):e2681. doi: 10.1371/journal.pntd.0002681 PMID: 24516683

25. Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Med Mal Infect. 2014 Jul; 44(7):302–7. doi: 10.1016/j.medmal.2014.04.008 PMID: 25001879

26. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, et al. Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus. PLoS Negl Trop Dis. 2016; 10(3):e0004543. doi: 10.1371/journal.pntd.0004543 PMID: 26938868

27. WHO. A review of Vectors of Zika–Aedes. Available from: http://who.int/features/2016/Aedes-Competency-ZIKAV.pdf.

28. Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: History, emergence, biology, and prospects for control. Antiviral Res. 2016; 130:69–80. doi: 10.1016/j.antiviral.2016.03.010 PMID: 26996139

29. Garcia E, Yactayo S, Nishino K, Millot V, Perea W, Brianda S. Zika virus infection: global update on epidemiology and potentially associated clinical manifestations. Wkly Epidemiol Rec. 2016; 91(7):73–81. doi: 10.1590/0074-02760160005162 PMID: 26897760

30. Noronha Ld, Zanluca C, Azevedo ML, Luz KG, Santos CN. Zika virus damages the human placental barrier and presents marked fetal neurotropism. Mem Inst Oswaldo Cruz. 2016; 111(5):287–93. doi: 10.1590/0074-02760160005162 PMID: 26897760

31. Musso D, Baud D, Gubler DJ. Zika virus: what do we know? Clin Microbiol Infect. 2016. pii: S1198-743X(16)30050-7.

32. Harbach RE. Culex pipiens: species versus species complex taxonomic history and perspective. J Am Mosq Control Assoc. 2012; 28(4 Suppl):10–23. PMID: 23401941

33. Bonaldo MC, Ribeiro IP, Lima NS, Santos AAC, Menezes LSR, Cruz SOD. Isolation of infective Zika virus from urine and saliva of patients in Brazil. PLoS Negl Trop Dis. 2016; 10(6):e0004816. doi: 10.1371/journal.pntd.0004816 PMID: 27341420

34. Vazeille M, Mousson L, Martin E, Failloux AB. Orally co-Infected Aedes albopictus from La Reunion Island, Indian Ocean, can deliver both dengue and chikungunya infectious viral particles in their saliva. PLoS Negl Trop Dis. 2010; 4(6):e706. doi: 10.1371/journal.pntd.0000706 PMID: 20540013

35. Hills SL, Russell K, Hennessey M, et al. Transmission of Zika virus through sexual contact with travelers to areas of ongoing transmission—continental United States, 2016. MMWR Morb Mortal Wkly Rep 2016; 65:215–6. doi: 10.15585/mmwr.mm6508e2 PMID: 26937739

36. Ayres CF. Identification of Zika virus vectors and implications for control. Lancet Infect Dis. 2016; 16(3):278–9. doi: 10.1016/S1473-3099(16)00073-6 PMID: 26852727

37. Carvalho RG, Lourenço-de-Oliveira R, Braga IA. Updating the geographical distribution and frequency of Aedes albopictus in Brazil with remarks regarding its range in the Americas. Mem Inst Oswaldo Cruz 2014; 109(6):787–96. PMID:25317707

38. Maciel-de-Freitas R, Vallee D. Challenges encountered using standard vector control measures for dengue in Boa Vista, Brazil. Bull World Health Organ. 2014; 92(9):685–9. doi: 10.2471/BLT.13.119081 PMID: 25378760

39. Lourenço-de-Oliveira R, Rio de Janeiro against Aedes aegypti: Yellow fever in 1908 and dengue in 2016. Mem Inst Oswaldo Cruz 2008; 103:627–8. PMID: 19057810

40. Maciel-de-Freitas R, Arendtsho FC, Santos R, Sylvestre G, Araujo SC, Lima JB, et al. Undesirable consequences of insecticide resistance following Aedes aegypti control activities due to a dengue outbreak. PLoS One. 2014; 9(3):e92424. doi: 10.1371/journal.pone.0092424 PMID: 24676277

41. Chediak M, G Pimenta F Jr, Coelho GE, Braga IA, Lima JB, Cavalcante KR, et al. Spatial and temporal country-wide survey of teneral mosquitoes in Brazilian populations of Aedes aegypti. Mem Inst Oswaldo Cruz. 2016; 111; 311–321. doi: 10.1590/0074-02760150409 PMID: 27143489

42. Dutra HL, Rocha MN, Dias FB, Mansur SB, Caragata EP, Moreira LA. Wolbachia Blocks Currently Circulating Zika Virus Isolates in Brazilian Aedes aegypti Mosquitoes. Cell Host Microbe. 2016; 19 (6):771–4. doi: 10.1016/j.chom.2016.04.021 PMID: 27156023
43. Fansiri T, Fontaine A, Diancourt L, Caro V, Thaisomboonsuk B, Richardson JH, et al. Genetic mapping of specific interactions between Aedes aegypti mosquitoes and dengue viruses. PLoS Genet. 2013; 9(8):e1003621. doi:10.1371/journal.pgen.1003621 PMID: 23935524

44. Lambrechts L, Chevillon C, Albright RG, Thaisomboonsuk B, Richardson JH, Jarman RG, et al. Genetic specificity and potential for local adaptation between dengue viruses and mosquito vectors. BMC Evol Biol. 2009; 9:160. doi:10.1186/1471-2148-9-160 PMID: 19589156

45. Lambrechts L. Quantitative genetics of Aedes aegypti vector competence for dengue viruses: towards a new paradigm? Trends Parasitol. 2011; 27(3):111–4. doi:10.1016/j.pt.2010.12.001 PMID: 21215699

46. Tabachnick WJ. Nature, nurture and evolution of intra-species variation in mosquito arbovirus transmission competence. Int J Environ Res Public Health. 2013; 10(1):249–77. doi:10.3390/ijerph10010249 PMID: 23343982

47. Aliota MT, Peinado SA, Osorio JE, Bartholomay LC. Culex pipiens and Aedes triseriatus mosquito susceptibility to Zika virus. Emerg Infect Dis. 2016; 22(10) [Epub ahead of print].

48. Ferreira-de-Brito A, Ribeiro IP, Miranda R, Fernandes RS, Campos SS, da Silva KA et al. First detection of natural infection of Aedes aegypti with Zika virus in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2016 E-pub: 22 Jul 2016. doi:10.1590/0074–02760160332

49. Guerbois M, Fernandez-Salas I, Azar SR, Danis-Lozano R, Alpuche-Aranda CM, Leal G et al. Outbreak of Zika virus infection, Chiapas State, Mexico, 2015, and first confirmed transmission by Aedes aegypti mosquitoes in the Americas. J Infect Dis pii: jiw302. [Epub ahead of print]