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Fernandes RS, Campos SS, Ribeiro PS, Raphael LMS, Bonaldo MC, Lourenço-de-Oliveira R. *Culex quinquefasciatus* from areas reporting the highest incidence of microcephaly associated to Zika virus infections, northeastern Brazil, are refractory to the virus [Submitted]. Mem Inst Oswaldo Cruz E-pub: 17 Ap 2017. doi: 10.1590/0074-02760170145.

Cx. quinquefasciatus and Zika virus

Culex quinquefasciatus from areas reporting the highest incidence of microcephaly associated to Zika virus infections, Northeastern Brazil, are refractory to the virus

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Abstract

Zika virus (ZIKV) is widely distributed in Brazil, with the Northeastern region (NE) the most affected zone with the highest incidence of microcephaly associated with ZIKV congenital infections. We report attempts to infect three populations of *Cx. quinquefasciatus* from severely affected sites from NE and Southeast (SE) regions of Brazil with three strains of ZIKV isolated from these localities. An *Ae. aegypti* population from SE, was used as a positive control. All tested *Cx. quinquefasciatus* populations were refractory to the ZIKV isolates. There is still no reason to consider *Cx. quinquefasciatus* as a potential vector of ZIKV in Brazil.

Key words: *Culex quinquefasciatus*, Zika virus, vector competence, Brazil.

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Financial support: CAPES-COFECUB Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (grant n. 799/14), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grants n. E-26/102.351/2013 and E-26/201.335/2016), Conselho Nacional Desenvolvimento Científico e Tecnológico (grant n. 309577/2013-6), National Institut of Health (grant n. 1UO1 AI115595-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript, European Union's Horizon 2020 research and innovation programme (ZIKAlliance grant agreement no. 734548).

After a rapid expansion in the Pacific region, Zika virus (ZIKV) was first recognized in Northeastern Brazil in 2015, followed by a countrywide epidemic that eventually spread to the entire continent (Zanluca et al. 2015, Possas et al. 2017). In 2016, 15,319 probable Zika cases were recorded in Brazil, and the Northeast (NE) region was the most affected zone, with the highest incidence (134.4/100,000 inhabitants) (http://portalsaude.saude.gov.br/index.php/o-inisterio/principal/secretarias/svs/boletim-epidemiologico#numerosrecentes). Moreover, it was in this Brazilian region where the first cases of microcephaly associated with ZIKV infection were reported, as well as having the highest incidence of this condition and other congenital neurological malformations worldwide. Indeed, 76.2% of the 2,366 confirmed cases of ZIKV-associated microcephaly recorded in Brazil in 2015-2016 occurred in this region of the country (Possas et al. 2017).

The primary vector of ZKV is *Aedes aegypti* (Weger-Lucarelli et al. 2016, Ferreira-de-Brito et al. 2016). However, due to its great abundance and anthropophilic behavior in epidemic areas, especially in low income districts where microcephaly was highest, *Culex quinquefasciatus* came under suspicion to be an alternative ZIKV vector. So, it was mandatory to investigate the ability of this species in terms of vector competence since it could be essential to ZIKV control. To date, experimental data with regard to *Cx. quinquefasciatus* vector competence for ZIKV has been somewhat contradictory. For instance, Guo et al. 2016 reported the detection of ZIKV RNA in saliva of orally infected Chinese *Cx. quinquefasciatus* and claimed this species to be a potential vector. In contrast, *Cx. quinquefasciatus* from Rio de Janeiro, Brazil, was shown to be unable to transmit local ZIKV isolates (Fernandes et al. 2016), a result also observed for United State of America and Australian populations exposed to several

ZIKV strains (Weger-Lucarelli et al. 2016, Hall-Mendelin et al. 2016, Hart et al. 2017). As vector competence is known to be geographically variable and depends on the specific combination of mosquito and virus genotypes (Lambrechts 2011, Tabachnick 2013), we challenged *Cx. quinquefasciatus* from two sites reporting high records of microcephaly associated to ZIKV infections in NE Brazil with three Brazilian ZIKV isolates from NE and Southeast (SE) regions.

We used F_1 generation of *Cx. quinquefasciatus* from NE, Brazil: Recife (Pernambuco State-PE- 08°03'14"S 34°52'52"W) and Campina Grande (Paraíba State-PB - 7°13′51″S 35°52′54″W). For comparison, we used two *Cx. quinquefasciatus* populations from SE: Manguinhos (F_1) and Triagem ($F_{>10}$), districts of Rio de Janeiro, whose low vector competence was previously determined for other ZIKV isolates (Fernandes et al. 2016), as well as an *Ae. aegypti* colony from Urca, Rio de Janeiro (F>10), which was previously shown to be a highly competent vector (Fernandes et al. 2016).

Mosquito rearing 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 Recife, Campina Grande and Rio de Janeiro.

All used ZIKV strains belong to the Asian lineage and were previously isolated from humans: ZIKVPE243 from the city of Recife, Pernambuco State, NE, Brazil (Donald et al. 2016), ZIKVSPH2015 from the city of Sumaré, São Paulo State, SE, Brazil (Faria et al. 2016), and ZIKV RioU-1 from Rio de Janeiro, SE, Brazil (Bonaldo et al. 2016). ZIKVSPH2015 has a high level of similarity with ZIKVPE243 (99.9% of nucleotides and 99.97% of amino acids) (Donald et al. 2016).

Female mosquitoes at 5-7 days post-emergence were fed with a mixture containing two parts of washed erythrocytes and one part of viral suspension through an artificial feeding apparatus. Depending on the availability, mosquitoes were examined on days 7, 14 and 21 post oral challenge (dpi). Homogenates of body (thorax + abdomen), and head were examined by plaque assays in culture of Vero cells for determining infection (IR) and dissemination rates (DR) and for *Culex* body sample we apply the real-time PCR reactions (RT-qPCR) technique to confirm positivity (for details see Fernandes et al. 2016). Saliva was also collected for examination if there were evidences of viral dissemination.

All tested *Cx. quinquefasciatus* were refractory to ZIKV regardless the viral strain (Table I). The body of only one out of 20 *Cx. quinquefasciatus* from Recife challenged with the ZIKVRioU1 was positive at 7 dpi, although the virus did not disseminate in this individual, as evidence by the head repeatedly testing negative. As the virus did not disseminate in any *Cx. quinquefasciatus*, saliva was not examined.

In contrast, all strains of ZIKV infected and disseminated in all *Ae. aegypti* populations, regardless the geographical origin of the isolates. Infection rates in *Ae. aegypti* ranged from 65% to 75% at 7 dpi and from 68% to 100% at 14 dpi; DR ranged from 86% to 100% at 14 dpi. This *Ae. aegypti* population (Urca) has previously exhibited high transmission rates (saliva infection) to two local ZIKV(Fernandes et al. 2016), and thus saliva of infected individuals were not exanimated here.

This is the first time that populations of *Cx. quinquefasciatus* from an area with high incidence of microcephaly and other congenital malformations associated with ZIKV infections, have been tested for vector competence to ZIKV from the same region. In agreement with results using SE Brazilian populations of *Cx.*

quinquefasciatus, they are incompetent to transmit the virus, including a strain isolated from the same epidemiological region. Our results of refractoriness to ZIKV are consistent with studies on this and other members of the *Culex pipiens* Complex (Fernandes et al. 2016, Weger-Lucarelli et al. 2016, Hall-Mendelin et al. 2016, Amraoui et al. 2016, Hart et al. 2017). The only exception is Guo et al. (2016) who reported the detection of ZIKV RNA in body and saliva of orally challenged Chinese *Cx. quinquefasciatus* mosquitos. Detection of residual RNA, crossing reactions and cross-contamination with positive control material, or other problems with molecular assays without adequate negative controls may explain this isolated discrepant result. Indeed, *Cx. quinquefasciatus* from several geographical origins and epidemiological situations has consistently shown to be refractory to ZIKV for both the African and Asian genotypes, even when challenged with blood meals with high viral titers (Fernandes et al. 2016, Hall-Mendelin et al. 2016, Hart et al. 2017).

Evidence from the current study and from previously published works, thus reinforce the conclusion that there is still no reason to consider *Cx. quinquefasciatus* as a potential vector of ZIKV. Even when the mosquito and ZIKV isolates are from localities with high incidence of human cases of Zika, *Cx. quiquefasciatus* is still an incompetent laboratory vector.

List of abbreviations

NE: Northeast region

SE: Southeast region

dpi: days post oral challenge

IR: Infection Rate

DR: Dissemination rate

RT-qPCR: quantitative real-time polymerase chain reaction

Authors' contributions

RSF and RLO conceived the study and wrote the manuscript. RSF and SSC carried out mosquito experimental infections and tested mosquito samples. LMSR and MCB produced the viral stocks and plaque assays for ZIKV titration. RPS performed mosquito collection and rearing in Recife and Campina Grande. All authors read and approved the final manuscript.

Acknowledgments

The authors thank Iule de Souza Bonelly, Rafaella Moraes de Miranda, Keli Antunes Barbosa da Silva and Anielly Ferreira-de-Brito for technical support. To Maria Ignêz Bersot for rearing some of the tested mosquito populations, and to Pedro Fernando da Costa Vasconcelos, Luciano Moreira and Marli T. Cordeiro for providing virus isolates. To Maria Goreti Rosa-Freitas and Jeffrey Powell for correcting the manuscript.

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Table 1. Infection (IR) and dissemination rates (DIR) of Brazilian *Culex quinquefasciatus* challenged with three ZIKV isolates.

Virus isolate/Origin/ Titer (PFU/ml)	**Mosquito Population**	**Days after exposure**	**Number examined per time point**	**IR per time point**	**DIR per time point**
ZIKVPE243 Recife, NE, 2015 $2.3x10^6$	*Cx. quinquefasciatus* Recife, NE (F1)	7,14	20,20	0,0	--
	Cx. quinquefasciatus Campina Grande, NE (F1)	7,14	20,20	0,0	--
	Cx. quinquefasciatus Rio de Janeiro, SE (>F10)	7,14	30,30	0,0	--
	Cx. quinquefasciatus Rio de Janeiro, SE (F1)	7,14	30,30	0,0	--
	Ae. aegypti Rio de Janeiro (>F10)	7,14	20,39	65%, 68%	30%, 86%
ZIKVSPH Sumaré, SE, 2015 $1.68x10^7$	*Cx. quinquefasciatus* Recife, NE (F1)	7,14	12,20	0,0	--
	Cx. quinquefasciatus Campina Grande, NE (F1)	7,14	20,20	0,0	--
	Cx. quinquefasciatus Rio de Janeiro, SE (>F10)	14,21	30,3	0,0	--
	Cx. quinquefasciatus Rio de Janeiro, SE (F1)	14,21	30,8	0,0	--
	Ae. aegypti Rio de Janeiro (>F10)	14	20	100%	100%
ZIKVU1 Rio de Janeiro, SE, 2015 $3.55x10^6$	*Cx. quinquefasciatus* Recife, NE (F1)	7,14	20,20	5%, 0%	0%, 0%
	Cx. quinquefasciatus Campina Grande, NE (F1)	7,14	20,20	0,0	--
	Ae. aegypti Rio de Janeiro (>F10)	7	20	75%	60%

212 DIR: Dissemination Rate refers to the proportion of mosquitoes with infected head among the infected mosquitoes;
213 IR: Infection Rate refers to the proportion of mosquitoes with an infected body (abdomen/thorax) among tested
214 mosquitoes; NE: Northeast region; SE: Southeast region.

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