



CES Medicina  
ISSN: 0120-8705  
ISSN: 2215-9177  
Universidad CES

Pérez-Pérez, Juliana; Rojo-Ospina, Raúl Alberto; Henao, Enrique;  
García-Huertas, Paola; Triana-Chavez, Omar; Rúa-Urbe, Guillermo  
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CES Medicina, vol. 33, no. 3, 2019, September-December, pp. 175-181  
Universidad CES

DOI: <https://doi.org/10.21615/cesmedicina.33.3.2>

Available in: <https://www.redalyc.org/articulo.oa?id=261162092003>

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## Investigación original

# Natural infection of *Aedes aegypti*, *Ae. albopictus* and *Culex spp.* with Zika virus in Medellin, Colombia

*Infección natural de Aedes aegypti, Ae. albopictus y Culex spp. con virus Zika en Medellín, Colombia*

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## Fecha correspondencia:

Recibido: marzo 28 de 2018.

Revisado: junio 28 de 2019.

Aceptado: julio 5 de 2019.

## Forma de citar:

Pérez-Pérez J, Rojo-Ospina RA, Henao E, García-Huertas P, Triana-Chavez O, Rúa-Urbe G. Natural infection of *Aedes aegypti*, *Ae. albopictus* and *Culex spp.* with Zika virus in Medellin, Colombia. Rev CES Med 2019. 33(3): 175-181.

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[Ética de publicaciones](#)

[Revisión por pares](#)

[Gestión por Open Journal System](#)

DOI: <http://dx.doi.org/10.21615/cesmedicina.33.3.2>

[cesmedicina.33.3.2](#)

ISSN 0120-8705

e-ISSN 2215-9177

Comparte



## Abstract

**Introduction:** The Zika virus has generated serious epidemics in the different countries where it has been reported and Colombia has not been the exception. Although in these epidemics *Aedes aegypti* traditionally has been the primary vector, other species could also be involved in the transmission. **Methods:** Mosquitoes were captured with entomological aspirators on a monthly basis between March and September of 2017, in four houses around each of the 250 entomological surveillance traps installed by the Secretaria de Salud de Medellin (Colombia). Additionally, 70 Educational Institutions and 30 Health Centers were visited each month. **Results:** 2 504 mosquitoes were captured and grouped into 1045 pools to be analyzed by RT-PCR for the detection of Zika virus. Twenty-six pools of *Aedes aegypti*, two pools of *Ae. albopictus* and one for *Culex quinquefasciatus* were positive for Zika virus. **Conclusion:** The presence of this virus in the three species and the abundance of these species in the different sectors of the city, suggests that the control strategies should be addressed to all species that could be potential vectors until the vectorial role of *Ae. albopictus* and *Culex quinquefasciatus* is completely elucidated in the transmission of the Zika virus.

**Keywords:** Zika, *Aedes*, *Culex*; Medellín.

## Resumen

El virus Zika ha generado serias epidemias en los diferentes países en donde se ha reportado, Colombia no ha sido la excepción. Aunque en estas epidemias el vector presuntamente involucrado ha sido *Aedes aegypti*, otras especies también podrían estar implicadas en su transmisión. **Metodología:** en el marco del Programa de vigilancia virológica en mosquitos de la Secretaría de Salud de Medellín (Colombia). **Resultados:** entre marzo y septiembre de 2017 se capturaron 2 504 mosquitos en predios visitados durante los levantamientos de índices entomológicos. El material entomológico fue agrupado en 1 045 pooles para ser analizados por RT-PCR para la detección de virus Zika. Resultaron positivos para virus Zika 26 pooles de *Aedes aegypti*, dos pooles de *Ae. albopictus* y uno para *Culex quinquefasciatus*. **Conclusión:** la presencia de este virus en las tres especies y su abundancia en los distintos sectores de la ciudad sugieren que las estrategias de control

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Although *Ae. aegypti* is considered the most important vector for the Zika virus transmission to humans, *Ae. albopictus* was associated as vector and recent studies have found the virus in *Culex* mosquitoes.

deban dirigirse a todos los potenciales vectores, hasta tanto no se dilucide completamente el papel vectorial de *Ae. albopictus* y *Cx. quinquefasciatus* en la transmisión del virus Zika.

**Palabras clave:** Zika, *Aedes*, *Culex*; Medellín.

**Introduction**

The Zika virus is a *Flavivirus* that recently caused serious epidemics in Brazil, Colombia and other countries of the continent. Between August 2015 and December 2016, 106.000 Zika cases were notified in Colombia (1). After Brazil, Colombia has been the most-affected country, with the highest number of probable cases reported during Zika virus outbreak in South America (2). Although it has been widely reported that approximately 80% of people with the virus infection are asymptomatic, Zika virus infection during pregnancy is a cause of microcephaly and other congenital abnormalities in the developing fetus and newborn and it may also be a trigger for Guillain-Barré syndrome in adults (3,4). In Colombia, 48% of the infants and fetuses with microcephaly reported after epidemic were positive for Zika virus infection (5).

This virus is transmitted to humans mainly by the bite of infected *Aedes* mosquitoes, but there is also evidence of infection by sexual transmission and congenital cases (6,7).

The first detection of the Zika virus in mosquitoes was in *Aedes africanus* (8). Then, the epidemic in the rural area of the Yap Islands in 2007 was attributed to *Ae. hensilli* (9) and *Ae. aegypti* was the main vector in the French Polynesia outbreak of 2013 (10).

Although *Ae. aegypti* is considered the most important vector for the Zika virus transmission to humans, *Ae. albopictus* was associated as vector in the Gabon outbreak (Central Africa) in 2007 (11) and recent studies have found the virus in *Culex* mosquitoes (12,13).

Despite the natural infection of mosquitoes with Zika virus has been reported, few studies have been carried out (14–17). The purpose of this study was to surveillance the presence of Zika virus in field-collected mosquitoes *Ae. aegypti*, *Ae. albopictus*, and *Culex spp* during a year after the epidemic in Colombia.

**Methods**

The study area was the city of Medellín (Antioquia - Colombia) which is the second largest in the country. Mosquitoes were captured with entomological aspirators on a monthly basis between March and September of 2017, in four houses in around each of the 250 entomological surveillance traps installed by the Secretaria de Salud de Medellín. Additionally, 70 Educational Institutions and 30 Health Centers were visited each month. Live mosquitoes were transported to the Medical Entomology Laboratory of the Faculty of Medicine of Universidad de Antioquia for taxonomic identification. Pools were formed containing one to 10 mosquitoes, which were analyzed by reverse transcription polymerase chain reaction (RT-PCR) for the detection of Zika virus.

Ribonucleic acid (RNA) extraction was performed using the commercial kit RNeasy Mini Kit® (Qiagen). Each pool was mechanically macerated following the protocol recommended by Qiagen. One-step RT-PCR was performed with the Luna Universal One-Step RT-qPCR kit® (Biolabs). Each 10µl reaction contained 1 µL of RNA, 1X enzyme mix, 1X reaction mix and 0.4 µM of each of the primers ZIKF-5'-CCTTGGATTCTTGAACGAGGA-3' and ZI-

KR-5'-AGAGCTTCATTCTCCAGATCAA-3' (18). These primers amplified a specific region of the NS5 gene of the virus of approximately 191 bp.

For the RT-PCR the following thermal profile was used: reverse transcription at 55°C for 10 min, initial denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 10s, 56°C for 30s and 72°C for 20s. Negative controls for extraction and amplification were included in each reaction, and virus RNA from the supernatant of infected cells was included as a positive control. The controls were processed simultaneously and under the same conditions as the samples. The amplification products were analyzed on 2.5% agarose gels in 0.5X Tris Borate EDTA (TBE), stained with Ethidium Bromide (0.6µg/ml) and visualized under (ultraviolet) UV light.

To confirm the results of the pools, positive samples were randomly selected and sequenced in Macrogen (Seoul, Korea). Forward and reverse sequences were manually edited and aligned using the BioEdit v.7.2.5 program. The sequences obtained were compared with a reference sequence (KY785466.1) from GenBank, which belongs to a Colombian isolate of the Zika virus, and were subsequently compared with the National Center for Biotechnology Information (NCBI) database using the Blastn algorithm to estimate the identity percentages with reported sequences of the Zika virus. This was a descriptive study conducted with full compliance with ethical standards of research. We explained the experimental procedure to the community and obtained consent from residents to collect mosquitoes. This study was classified as risk-free research according to resolution number 8340 (1993) from the Ministerio de Salud y Protección Social of Colombia.

From the 1 045 pools evaluated, 2.8 % were found to be positive for Zika virus.

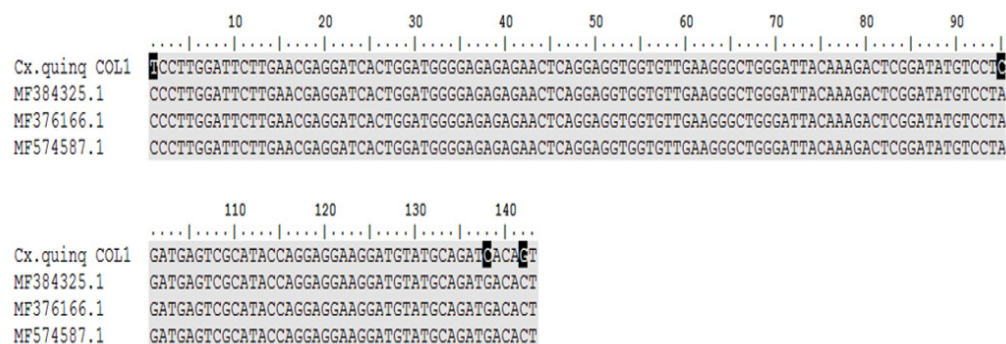
## Results

A total of 2504 mosquitoes were analyzed (*Ae. aegypti*, *Ae. albopictus* and *Culex spp.*). A subset of mosquitoes was dissected to separate the abdomen section and the secondary tissues such as the head and thorax to identify the disseminated form of the virus. From the 1 045 pools evaluated, 2.8 % were found to be positive for Zika virus (Table 1).

**Table 1.** Number of mosquitoes collected and number of analyzed pools for detection of Zika virus

<i>Specie</i>	<i>No. of females tested</i>	<i>No. of males tested</i>	<i>No. pools tested</i>	<i>No. of positive pool for ZIKV (%)</i>
<i>Ae. aegypti</i>	1119	1014	839	26 (2.5)
<i>Ae. albopictus</i>	75	53	78	2 (0.2)
<i>Culex spp</i>	175	68	128	1(0.1)
<b>Total</b>	<b>1369</b>	<b>1135</b>	<b>1045</b>	<b>28</b>

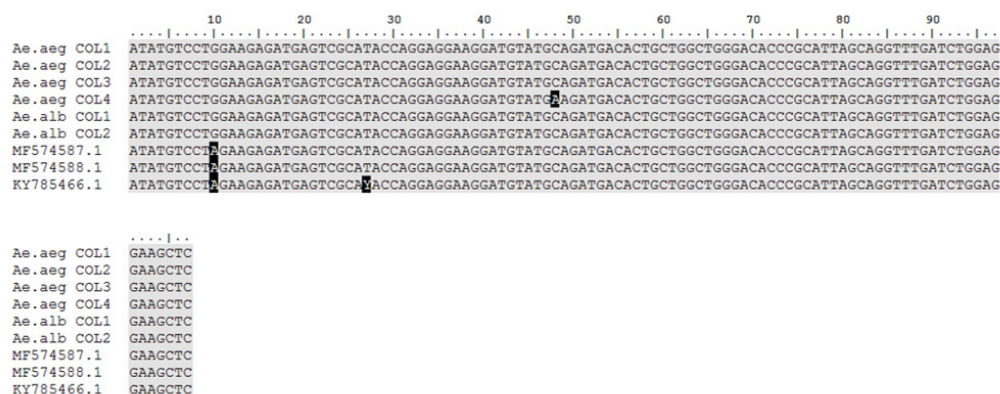
The sequences obtained presented identity values greater than 96%. The nucleotide sequence of the *Cx. quinquefasciatus* pool showed high identity percentage with the isolates from Haiti (MF384325.1), Brazil (MF376166.1) and Colombia (MF574587.1) (Figure 1).



**Figure 1.** Nucleotide sequence alignment of Zika virus found in *Cx. quinquefasciatus*

The MF384325.1 sequence correspond to isolate from Haiti, MF376166.1 sequence correspond to isolate from Brazil; and MF574587.1 sequence to isolate from Colombia.

On the other hand, the nucleotide sequences of *Ae. albopictus* and *Ae. aegypti* presented identity percentages around 98% with isolates from Colombia (MF574587.1, MF574588.1 and KY785466.1) (Figure 2).



**Figure 2.** Nucleotide sequence alignment of Zika virus found in *Ae. albopictus* and *Ae. aegypti*

The MF574587.1, MF574588.1 and KY785466.1 sequences correspond to isolates from Colombia

This is the first report of natural infection of Zika virus in *Aedes* and *Culex* mosquitoes in Colombia.

## Discussion

This is the first report of natural infection of Zika virus in *Aedes* and *Culex* mosquitoes in Colombia. Similar results for *Aedes* mosquitoes have been registered in Malaysia (14) and recently in Senegal (15), Brazil (17) and Mexico (19). For the case of *Cx. quinquefasciatus*, there is controversy with the incrimination of this mosquito as Zika vector. Some laboratory research has shown that this species is not competent to transmit the virus (17,20–22), but recent studies have concluded that *Cx. quinquefasciatus* could have a role in the transmission. In Brazil, the presence of the virus was detected in mosquitoes captured in the areas of incidence of the disease and in laboratory conditions, artificially-fed *Cx. quinquefasciatus* mosquitoes were able to replicate the virus in the midgut, salivary glands and saliva (13). These results are similar to those previously reported in China (12).

It is important to consider that, according to the feeding habits of *Cx. quinquefasciatus*, it is possible that our results are due to recent feeds on viraemic hosts. Therefore, future studies are necessary to determine the vectorial competence of this species in the transmission of the Zika virus.

Regarding the infection with Zika virus in *Ae. albopictus*, it has been previously demonstrated that this species is a competent vector for the virus under laboratory conditions (23,24), and is considered the main vector in some places like Gabon (11) and Sinagapur (25).

Our results do not allow the incrimination as vector of *Ae. albopictus* in the transmission of Zika. However, finding it naturally infected in head and thorax, highly suggests that it plays a role in the transmission of this arbovirolosis. Therefore, the monitoring of this species should be considered in anti-vector programs and subsequent studies should be conducted to determine its vector role in the transmission of Zika and new arboviruses.

## Conclusion

The results obtained in this study evidence the importance of the virological surveillance of mosquitoes and the need to incorporate it into public control programs. This also implies a redesign of the surveillance and control strategies for arbovirolosis, due to the differences in the bionomies of these three mosquito species. These findings show the need to monitor of virus in mosquitoes in other areas of the country, which allow to determine the vectorial role of these species in Colombia.

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## Acknowledgements

Many people and Institutions have contributed to this research. We are grateful for the support by COLCIENCIAS (project code: 111574455690. Contract number 634-2017) and the Secretaria de Salud de Medellín for the support and assistance with field work during this project. Also, we recognize the assistance of the Grupo Entomología Médica (GEM) and Grupo de Biología y Control de Enfermedades Infecciosas (BCEI) of the Universidad of Antioquia for the contributions to this work. Finally, and most importantly, we would like to thank the people of Medellín for welcoming us in to their homes to conduct this sampling work.

## Conflict of interest

The authors declare that they have no conflict of interests.

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