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# ENTOMOLOGICAL INVESTIGATION FOLLOWING A ZIKA OUTBREAK IN BROWNSVILLE, TEXAS

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## Abstract

In November and December 2016, an outbreak of locally transmitted Zika occurred in Brownsville, TX. The Texas Department of State Health Services requested for a Centers for Disease Control and Prevention (CDC) Epi Aid, and as part of that Epi Aid a team of CDC entomologists was deployed in January 2017. The mission was to improve mosquito-based arbovirus surveillance and evaluate the possibility of continuing local Zika virus (ZIKV) transmission in the city. The mosquito-based arbovirus surveillance program was expanded from 4 to 40 BG-Sentinel traps evenly distributed throughout the city. Over a 2-wk period, 15 mosquito species were detected; the most abundant species were *Culex quinquefasciatus, Aedes aegypti,* and *Ae. albopictus,* which accounted for 66.7%, 16.2%, and 5.7% of the total mosquito collection, respectively. The relative abundance of *Ae. aegypti* (1.0 mosquitoes/trap/day) and *Ae. albopictus* (0.4 mosquitoes/trap/day) was very low and unlikely to initiate and/or sustain ZIKV transmission. Zika virus was not detected in the mosquitoes collected, suggesting no or extremely low ZIKV transmission at that time.

### Keywords

Zika; outbreak; Brownsville; Texas; *Aedes aegypti* ; *Aedes albopictus*

Zika virus (ZIKV) (Family *Flaviviridae,* Genus Flavivirus) was first isolated in Zika Forest, Uganda, in 1947 (Dick et al. 1952). In the following years, a number of ZIKV strains were isolated in and around Zika Forest (Weinbren and Williams 1958, Haddow et al. 1964), and this suggested that ZIKV is endemic in this area. During the same time period, ZIKV antibodies were detected in human sera in different parts of Africa and Asia (Dick 1952, Smithburn 1952, MacNamara 1954, Smithburn et al. 1954, Hammon et al. 1958), which suggested widespread human infections. However, the first confirmed ZIKV human case was a probable laboratory infection in Entebbe, Uganda, in 1964 (Simpson 1964). The first documented large ZIKV outbreak was on the Island of Yap in the Federated States of Micronesia, in 2007 (Duffy et al. 2009). Subsequently, large outbreaks were reported in French Polynesia, and in South and Central America (Cao-Lormeau et al. 2014, Musso et al. 2014, Robbiani et al. 2017, Zambrana et al. 2018).

In the continental USA outbreaks of locally transmitted ZIKV were first detected in the cities of Wynwood and Miami Beach, both in Miami-Dade County, FL, in 2016 (Likos et al. 2016). On November 28, 2016, the Texas Department of State Health Services (Texas DSHS) reported its first confirmed locally transmitted case of Zika in the city of Brownsville, TX (https://www.dshs.texas.gov/news/releases/2016/20161128.aspx). Brownsville is in southern Texas along the USA–Mexico border adjacent to the city of Matamoros, Mexico. The Texas DSHS reported 4 more cases on December 9, 2016 (https://dshs.texas.gov/news/releases/2016/20161209.aspx), and another case on December 22, 2016 (https://dshs.texas.gov/news/releases/2016/20161222.aspx). In mid-January 2017, a Centers for Disease Control and Prevention (CDC) vector team was deployed to Brownsville, TX, for 2 wk: January 10–24, 2017. The overall mission was to provide vector assistance to the city of Brownsville. The CDC entomologists worked with the city of Brownsville vector control program to improve mosquito-based arbovirus surveillance. In the process, information was obtained on basic entomological parameters in Brownsville.

Before the deployment of CDC entomologists, the Brownville mosquito-based arbovirus surveillance program consisted of 4 BG-Sentinel Traps (BioQuip, Rancho Dominguez, CA) and 9 CDC gravid traps (Fig. 1A) monitored 3 days a week. At that time BG-Sentinel traps were in short supply and traps were deployed as they became available: from January 12–16, 2017, 18 BG-Sentinel traps were used; from January 16–18, 2017, the number increased to 22 BG-Sentinel traps; and from January 18–23, 2017, a total of 40 BG-Sentinel traps were used (Fig. 1B). The mosquito populations were sampled daily for 11 days, January 12–23, 2017. The BG Sentinel traps were baited with a BG-Lure (BioQuip) and dry ice as a source of $CO_2$. The traps were operated 24 h per day and the mosquitoes captured were collected each morning. The captured mosquitoes were transported to the laboratory in coolers on ice packs to minimize mortality and to maximize the survival of arboviral agents. In the laboratory, the mosquitoes were identified to species based on morphological characters by using the keys of Darsie and Ward (2005). *Aedes* (*Stegomyia*) *aegypti* (L.) and *Ae.* (*Stegomyia*) *albopictus* (Skuse) were pooled separately, placed in labeled tubes (labeled with mosquito species, sex, number of specimens, site and date of collection), and shipped to the Arbovirus-Entomology Laboratory at the Texas DSHS in Austin, TX, for arbovirus screening. In the laboratory, *Ae. aegypti* and *Ae. albopictus* pools were tested for ZIKV virus, using a real-time reverse transcriptase–polymerase chain reaction (RT-PCR) assay (Lanciotti et al. 2008). Briefly, pools were homogenized in diluent, containing 1% bovine serum albumin and antibiotics (gentamicin sulfate and amphotericin B). After clarification by centrifugation, RNA was extracted, using the QIAamp Viral RNA Mini Kit (catalog no. 52904; Qiagen, Germantown, MD) per the manufacturer's instructions, with the final elution in 60 µl of nuclease-free water. The RT-PCR was performed, using the SuperScript III Platinum One-Step qRT-PCR Kit (catalog no. 11732–088; Invitrogen, Grand Island, NY) with 5 µl of sample RNA in a 25-µl reaction volume. The Zika1087/Zika1163c primers and

Zika1108FAM probe were used, and the aim was to detect all known ZIKV genotypes as described by Lanciotti et al. (2008).

A total of 199 mosquito pools were tested for ZIKV, consisting of 149 female *Ae. aegypti* pools (*n* = 344 mosquitoes), 2 male *Ae. aegypti* pools (*n* = 3 mosquitoes), 41 female *Ae. albopictus* pools (*n* = 123 mosquitoes), and 7 female *Aedes* spp. pools (*n* = 11 mosquitoes). All mosquito pools tested were negative for ZIKV.

Fifteen different mosquito species in 6 different genera—*Aedes* (4 species), *Anopheles* (4 species), *Culex* (3 species), *Culiseta* (1 species), *Mansonia* (1 species), and *Uranotaenia* (1 species)—were detected from a total of 2,149 mosquito specimens collected (Table 1). Three species, *Anopheles atropos* Dyar and Knab, *Culex erythrothorax* Dyar, and *Ae. vexans* (Meigen), were not included in the species lists of the city of Brownsville by Fisk and Le Van (1940) and Srinivasan et al. (2017), and to our knowledge this is the 1st detection of these species in this city. *Anopheles atropos* is a saltwater mosquito restricted to narrow margins of the Gulf and Atlantic coasts. The distribution map published by Darsie and Ward (2005) stopped north of Brownsville, suggesting that Brownsville was not within the distribution range despite the abundant suitable larval sites within the city. Only a single specimen was detected (Table 1), suggesting very low relative abundance of this species in Brownsville. The distribution range of *Cx. erythrothorax* extends from the West and the Southwest of the USA to northern Texas (Darsie and Ward 2005). The detection reported in this note represents an expansion of that range. *Aedes vexans* is widely distributed throughout North America (Darsie and Ward 2005), and it is not clear why it has not previously been detected in Brownsville. The fact that we detected only a single specimen suggests that it is rare in Brownsville, and that is probably why it was not detected by Fisk and Le Van (1940) and Srinivasan et al. (2017). However, all 3 species have previously been detected in Texas and do not represent new introductions to the state.

The most common species were *Cx. quinquefasciatus* Say (66.7% of the total collection), *Ae. aegypti* (16.15%), and *Ae. albopictus* (5.7%) (Table 1); all the other 12 species combined were less than 12% of the total collection. The average abundance for the most common species over the entire sampling period was 3.36 mosquitoes/trap/day for *Cx. quinquefasciatus,* 1.0 mosquitoes/trap/day for *Ae. aegypti,* and 0.4 mosquitoes/trap/day for *Ae. albopictus*. The relative abundance estimates for the 3 most common species varied over the 11-day sampling period between 0.5 and 6.6 mosquitoes/trap/day for *Cx. quinquefasciatus,* 0.4–2.9 mosquitoes/trap/day for *Ae. aegypti,* and from 0.1 to 0.8 mosquitoes/trap/day for *Ae. albopictus* (Fig. 2). Interestingly, male mosquitoes were detected throughout the sampling period, suggesting continuous mosquito production in the wintertime in Brownsville, TX. Overall, the relative abundance of the ZIKV vector species was very low; the abundance of *Ae. albopictus* was consistently below 1 mosquito/trap/day and that of *Ae. aegypti* was below 2 mosquitoes/trap/day except on January 17, 2017, when it was 2.7 mosquitoes/trap/day (Fig. 2).

One of the aims of our study was to evaluate the possibility of continued transmission of ZIKV in Brownsville. Based on our observations the population sizes of the ZIKV vectors at that time, 1.0 mosquitoes/trap/day for *Ae. aegypti* and 0.4 mosquitoes/trap/day for *Ae. albopictus,* were not high enough to initiate and/or sustain ZIKV transmission. The population sizes associated with arbovirus transmission in Puerto Rico were above 2–3 mosquitoes/trap/day (Barrera et al. 2017), and our studies showed that the population levels of *Ae. aegypti* and *Ae. albopictus* in Brownsville were below that threshold. Furthermore, ZIKV was not detected in the mosquitoes processed, suggesting that either ZIKV was circulating in extremely low levels at that time or that there was no ZIKV transmission.

The 15 species detected included 10 species that have been associated with arbovirus transmission. The detection of a wide variety mosquito species that are vectors of arbovirus of public health importance suggests a high potential for arboviral transmission and outbreaks in Brownsville, TX. The fact that we detected these vector species in January, during wintertime, suggests potential for arbovirus transmission the whole year-round. Some of these vector species were detected by Fisk and Le Van (1940) and by Srinivasan et al. (2017), and this suggests that these species have been established in Brownsville for a long time and that the potential for arbovirus transmission in Brownsville is not a recent development. This is supported by a long history of documented arboviral disease outbreaks in Brownsville (Hughes and Porter 1958, Duffy 1968, Brunkard et al. 2007, Ramos et al. 2008, Hall et al. 2017). The mosquito surveillance system we established was maintained and has been improved to 52 permanent traps.

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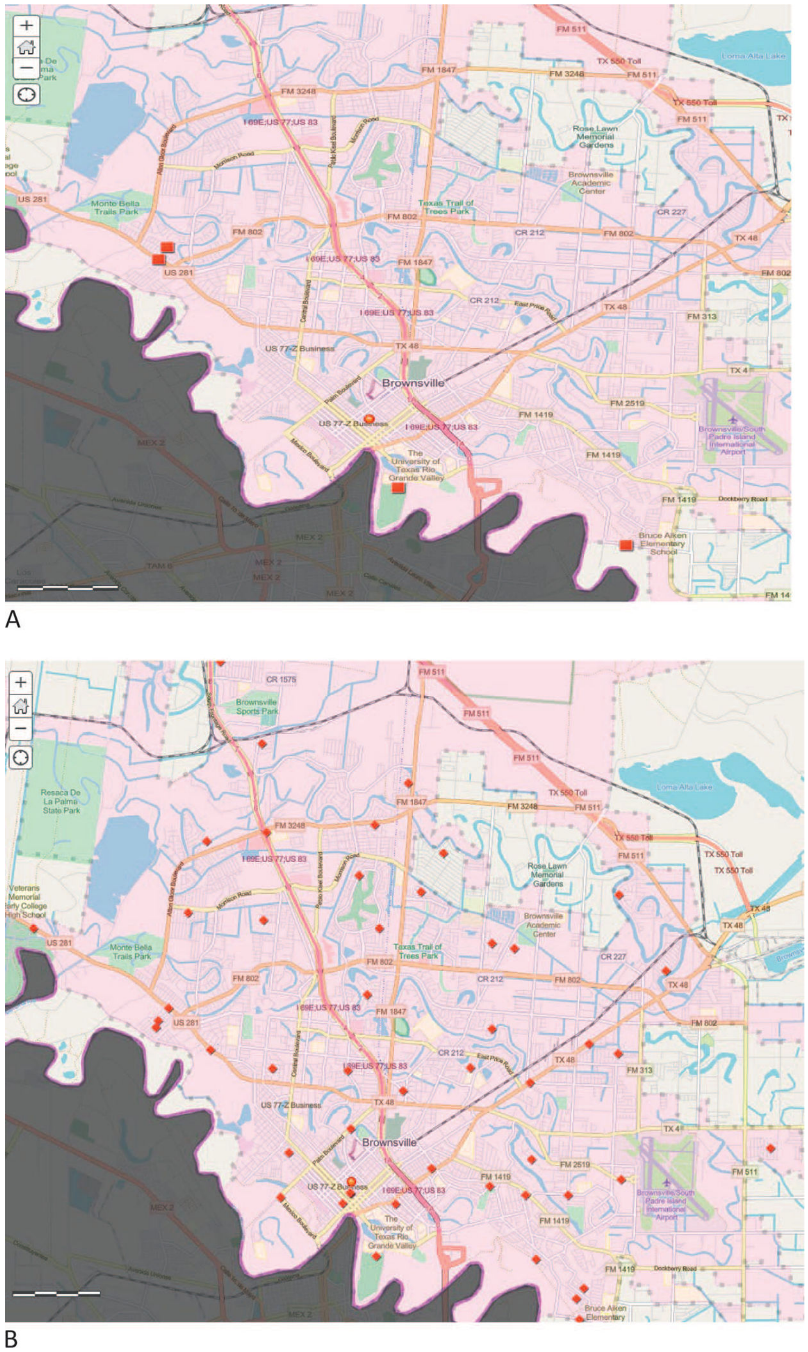


**Fig. 1.**

Mosquito trap location in Brownsville, TX, (A) before and (B) after expanding surveillance.

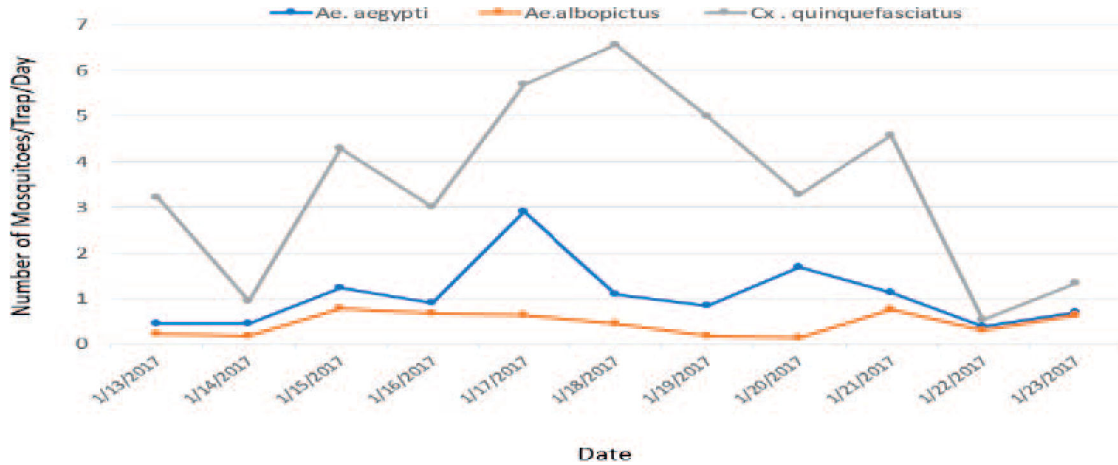


**Fig. 2.**

The relative abundance of *Culex quinquefasciatus, Aedes aegypti,* and *Ae. albopictus* in Brownsville, TX.

## Table 1.

Mosquito species collected in Brownsville, TX, in January 2017.

| Species | Number collected (%) |
|---|---|
| *Culex quinquefasciatus* | 1,434 (66.73) |
| *Aedes aegypti* | 347 (16.15) |
| *Ae. albopictus* | 123 (5.72) |
| *Cx. coronator* | 50 (2.33) |
| *Cx. erythrothorax* | 15 (0.70) |
| *Cx. tarsalis* | 10 (0.47) |
| *Anopheles pseudopunctipennis* | 7 (0.33) |
| *An. punctipennis* | 2 (0.09) |
| *Ae. sollicitans* | 1 (0.05) |
| *Ae. vexans* | 1 (0.05) |
| *An. albimanus* | 1 (0.05) |
| *An. atropos* | 1 (0.05) |
| *Culiseta inornata* | 1 (0.05) |
| *Mansonia titillans* | 1 (0.05) |
| *Uranotaenia iowii* | 1 (0.05) |
| *Culex* spp. | 92 (4.28) |
| *Anopheles* spp. | 46 (2.14) |
| *Aedes (Stegomyia)* spp. | 14 (0.65) |
| *Aedes* spp. | 1 (0.05) |
| *Uranotaenia* spp. | 1 (0.05) |
| Total | 2,149 |