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ENTOMOLOGICAL INVESTIGATION OF *AEDES AEGYPTI* IN NEIGHBORHOODS WITH CONFIRMED HUMAN ARBOVIRUS INFECTION IN PUERTO RICO

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Abstract

The exotic arboviruses chikungunya (CHIKV) and Zika (ZIKV) recently caused large outbreaks and continue to circulate in Puerto Rico, prompting entomological investigations at 9 locations with confirmed CHIKV- or ZIKV-infected human cases. Adult mosquitoes were collected using the Centers for Disease Control and Prevention autocidal gravid ovitraps over a 14-day period at each site. Mean female *Aedes aegypti* captured per trap-week ranged from 13.47 per trap-week to 1.27 per trap-week. Arbovirus-positive pools were detected at 7 of the 9 sampling sites. We investigated vertical transmission by collecting *Ae. aegypti* eggs in a single location where ZIKV was found in adult mosquitoes. We discuss the relationship between vector density and infection rates and its implications for determining mosquito density thresholds of novel invasive arboviruses such as CHIKV and ZIKV.

Keywords

Autocidal gravid trap; *Aedes aegypti*; chikungunya virus; Puerto Rico; Zika virus

Exotic arboviruses, chikungunya virus (CHIKV; *Alphaviridae, Alphavirus*) and Zika virus (ZIKV; *Flaviviridae, Flavivirus*), recently emerged in Puerto Rico. In absence of natural immunity in human populations, the introduction of these viruses resulted in outbreaks of chikungunya (CHIK) and Zika (ZIK) in 2014 and 2016, respectively (Adams et al. 2016, Sharp et al. 2016). Both of these arboviruses are transmitted between humans by the bite of *Aedes* species in urban areas (Tan et al. 2011). In December 2013 the 1st autochthonous case of CHIK was reported on the Caribbean island of Saint Martin (Fischer and Staples 2014). The virus subsequently spread through the Caribbean and into South, Central, and North America (Fischer and Staples 2014). Since its detection in Puerto Rico in May 2014, there were 28,327 suspected cases reported from all municipalities (8.0 per 1,000 residents; PRDH 2015). The 1st report of autochthonous mosquito-transmitted ZIKV in the Americas was in Brazil in early 2015; by the end of the year an additional 26 countries and territories reported cases, including Puerto Rico in December 2015 (Zanluca et al. 2015, Thomas et al.

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2016). During the 1st year of the outbreak, there were 36,249 confirmed cases across all 78 municipalities (PAHO 2017).

In order to expand the current understanding of newly introduced arbovirus transmission in Puerto Rico, we conducted entomological studies to quantify the density and presence of CHIKV and ZIKV in *Aedes aegypti* (L.) during outbreaks associated with confirmed human cases of CHIK and ZIK. Nine sites from 8 neighborhoods were sampled in 2014 and 2016 using the Centers for Disease Control and Prevention autocidal gravid ovitraps (AGO) (Barrera et al. 2014a, 2014b). All neighborhoods were categorized as suburban, consisting of single-story homes, except for Villa del Sol, which was a multibuilding, 2-story apartment complex. At each site, 30 AGOs were uniformly deployed within a 200-m radius of the index case's house on an average of 22 days after symptom onset. The AGOs were inspected for mosquitoes every 4 or 5 days over a period of 14 days at each site. Captured mosquitoes were removed using tissue probes and forceps, and sexed. Female *Ae. aegypti* mosquitoes were pooled by species, placed in 2.0-ml vials containing 10–20 specimens on ice, and stored in the laboratory at −80°C until the samples could be analyzed for the presence of CHIKV, ZIKV, and dengue viruses (DENV) 1–4 ribonucleic acids by real-time reverse transcription–polymerase chain reaction (RT-PCR) (Barrera et al. 2017, FDA 2017, Paz-Bailey et al. 2017).

During these investigations, 3,246 females of *Ae. aegypti* were collected and tested for arbovirus infection (Table 1). Sampling occurred throughout the CHIK outbreak, and early during the ZIKV transmission period (Fig. 1). The highest number of female *Ae. aegypti* was recorded in El Coco, Salinas (13.47 per trap-week), and the lowest was at Lirios, Juncos (1.27 per trap-week) (Table 1). Few *Aedes* (*Gymnometopa*) *mediovittatus* (Coq.) ($N = 23$) were captured throughout the investigation, and no mosquito pools tested positive for arboviruses. Overall, virus-positive pools of female *Ae. aegypti* were detected at 7 of 9 sample sites; 3 of 4 sites sampled for CHIKV in 2014 were positive for the virus, and 4 of the 5 sites sampled in 2016 were positive for ZIKV (Table 1). Zika virus was not present in Puerto Rico in 2014; hence, samples were not tested for ZIKV during this period. A single pool positive for DENV type 3 was detected in El Coco, Salinas, in 2014. Maximum likelihood minimum infection rates of mosquitoes were calculated using PooledInfRate version 4.0 (Biggerstaff 2016). The overall infection rate during the 2014 CHIKV outbreak was 8.9 per 1,000 and ranged from 6.9 to 12.1 per 1,000. During the 2016 ZIKV outbreak, the infection rate across all sampling sites was 9.0 per 1,000 and ranged from 4.7 to 18.7 per 1,000 (Table 1).

Concurrent with the entomological investigation in Buenaventura, Carolina, 30 large ovicups (4.8 liter) were placed adjacent to the AGOs from March 10 to March 14, 2016. A total of 11,602 eggs were collected and identified as *Ae. aegypti* and reared under standard laboratory conditions (25–27°C, 75% RH, and a 12:12 light:dark cycle), of which 6,297 emerged as adults (3,006 females and 3,291 males). From these, 500 (17%) females and 500 (15%) males were aspirated from a common rearing cage for convenience and killed by freezing. Mosquitoes were combined into pools of 10 and stored at −80°C until they could be tested for the presence of ZIKV in mosquitoes by real-time RT-PCR. None of the 100 pools tested were positive for ZIKV.

In the Americas, entomological investigations reported CHIKV infection rates of 8.8 and 23.3 per 1,000 mosquitoes in Guerrero and 32.3 per 1,000 in Chiapas, Mexico (Diaz-Gonzalez et al. 2015, Dzul-Manzanilla et al. 2015). An investigation in the Union of Comoros in 2005 found a single CHIKV-infected pool of *Ae. aegypti* with an infection rate of 4.0 per 1,000, and on Yap Island of the Federated States of Micronesia the reported infection rate was 70 per 1,000 (Sang et al. 2008, Savage et al. 2015). In Mexico, ZIKV infection rates in *Ae. aegypti* ranged from 52.5 to 172.7 per 1,000 (Guerbois et al. 2016). Our findings are not directly comparable to these studies due to methodological differences. For example, backpack aspirators and mosquito landing collections were used in the Mexico and Comoros studies, while this study used AGOs, which are designed to attract and collect ovipositing females. Infection rates of gravid mosquitoes captured in AGOs are expected to be greater than those captured using Biogents Sentinel traps or with mosquito landing collections, as gravid females have already taken at least 1 blood meal that may have been infectious. Infection rates of CHIKV in this study were higher than in a previous study in Puerto Rico using AGOs that showed an overall infection rate of 2.4 with a range from 0 to 38 per 1,000 mosquitoes, and densities of *Ae. aegypti* ranged from 1.2 to 17.4 females per trap-week (Barrera et al. 2017).

These findings examined the relationship between mosquito density and mosquito infection rate in Puerto Rico soon after the introduction of these exotic arboviruses. Entomological investigations of human diseases often attempt to determine a minimum threshold of mosquito density needed for sustained virus transmission. Determining a threshold of mosquito density is important because, below such a threshold, transmission of arboviruses may be unsustainable. Furthermore, a quantified threshold would provide vector control personnel a target for mosquito control. In practice, the threshold is difficult to estimate because of biases inherent to mosquito traps, heterogeneity in mosquito density, as well as heterogeneity in available human hosts that are susceptible to infection. Entomological investigations in Australia found that after vector control initiation, local transmission of DENV ceased and sticky ovitrap collections dropped to <0.5 mosquitoes per trap per week (Ritchie et al. 2004). Investigations conducted in southern Puerto Rico, where AGOs were used for vector control purposes, showed that people from most homes that were using 3 traps per home were 50% less likely to have CHIKV IgG antibodies. The density of *Ae. aegypti* in those neighborhoods was <3 females per trap per week in sentinel AGOs (Lorenzi et al. 2016, Barrera et al. 2017). In Brazil, a mean female *Aedes* index (an average of the mean number of female mosquitoes captured per week, over the 3 previous weeks) of <0.2 in the MosquiTrap was considered below the threshold required for sustained DENV transmission (Eiras and Resende 2009).

The sites with the lowest mean captures of *Ae. aegypti* were Villa del Sol, Trujillo Alto (4.84 per trap-week), Lirios, Juncos (1.27 per trap-week), Buenaventura, Carolina (4.08 per trap-week), and Campamento, Gurabo (4.81 per trap-week). Interestingly, 2 of these sites, Villa del Sol and Campamento, did not have any positive pools. Finding fewer mosquitoes at sites without virus-positive pools is encouraging but does not necessarily reveal a threshold density for arbovirus transmission. For example, in Buenaventura and Lirios, the mean *Ae. aegypti* captured were 4.08 and 1.27 per 1,000, respectively, and both sites had at least 1 virus-positive pool. Barrera et al. (2017) reported that local transmission of CHIKV was

more likely when *Ae. aegypti* abundance was >3 mosquitoes per trap-week. Under these conditions, Lirios would be an outlier, assuming that sustained local transmission is unlikely when only a single virus-positive pool is detected in an area of low mosquito density. The possibility that this finding was the result of recent virus reintroduction due to movement of infected people cannot be excluded.

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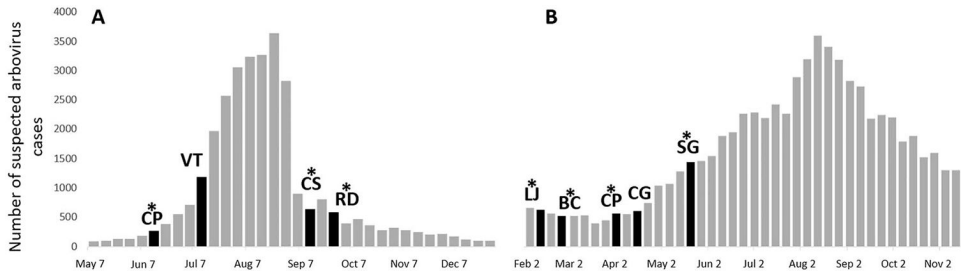

Fig. 1.

Epidemiological curves from Puerto Rico with mosquito sampling periods: (A) 2014 chikungunya and (B) 2016 Zika. Black bars indicate when autocidal gravid traps were deployed in neighborhoods, and an asterisk (*) indicates if virus-positive pools were detected. In 2014 we sampled locations Cantera, Ponce (CP); Villa del Sol, Trujillo Alto (VT); El Coco, Salinas (CS); and Rio Lajas, Dorado (RD); and in 2016 we sampled locations Lirios, Juncos (LJ); Buenaventura, Carolina (BC); Cantera, Ponce; Campamento, Gurabo (CG); and Savarona, Caguas (SG).

Table 1.

Surveillance of chikungunya virus (CHIKV) and Zika virus (ZIKV) in female *Aedes aegypti* by reverse transcription–polymerase chain reaction in neighborhoods with confirmed arbovirus infections during the 2014 and 2016 epidemics in Puerto Rico.

Location	Date	No. female *Ae. aegypti* collected	Mean female *Ae. aegypti* per trap-week (95% CI)	No. pools tested (avg. size)	CHIKV-positive pools (%)	CHIKV infection rate per 1,000 (95% CI)	ZIKV-positive pools (%)	ZIKV infection rate per 1,000 (95% CI)
Cantera, Ponce	Jun. 15, 2014	371	6.4 (4.95–7.84)	20 (20)	4 (20)	11.4 (3.7–27.6)	NT[1]	—
Villa del Sol, Trujillo Alto	Jul. 12, 2014	281	4.84 (3.94–5.74)	33 (9)	0 (0)	—	NT	—
El Coco, Salinas[2]	Sept. 10, 2014	781	13.47 (11.58–15.35)	80 (10)	8 (10)	12.1 (5.9–22.0)	NT	—
Rio Lajas, Dorado	Sept. 28, 2014	441	7.60 (6.29–8.92)	48 (9)	3 (6)	6.9 (1.8–18.5)	NT	—
Lirios, Juncos	Feb. 12, 2016	76	1.27 (0.97–1.56)	10 (10)	0	—	1 (10)	13.7 (0.8–67.1)
Buenaventura, Carolina	Feb. 29, 2016	245	4.08 (3.33–4.83)	27 (9)	0	—	3 (11)	12.5 (3.4–33.5)
Cantera, Ponce	Apr. 5, 2016	428	7.13 (6.11–8.16)	47 (9)	0	—	2 (4)	4.7 (0.9–15.5)
Campamento, Gurabo	Apr. 22, 2016	279	4.81 (3.9–5.72)	29 (10)	0	—	0 (0)	—
Savarona, Caguas	May 26, 2016	344	6.14 (5.19–7.1)	35 (10)	0	—	6 (17)	18.7 (7.7–38.6)

[1] NT indicates not tested.

[2] A single pool ($N = 1$, 1%) from El Coco, Salinas, was positive for dengue virus type 3.

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