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Susceptibility and Vectorial Capacity of American *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) to American Zika Virus Strains

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Abstract

The rapid expansion of Zika virus (ZIKV), following the recent outbreaks of Chikungunya virus, overwhelmed the public health infrastructure in many countries and alarmed many in the scientific community. *Aedes aegypti* (L.) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) have previously been incriminated as the vectors of these pathogens in addition to dengue virus. In our study, we challenged low generation *Ae. aegypti* (Chiapas, Mexico) and *Ae. albopictus* (North Carolina, Mississippi), with three strains of ZIKV, Puerto Rico (GenBank: KU501215), Honduras (GenBank: KX694534), and Miami (GenBank: MF988743). Following an oral challenge with $10^{7.5}$ PFU/ml of the Puerto Rico strain, we observed high infection and dissemination rates in both species (95%). We report estimated transmission rates for both species (74 and 33%, for *Ae. aegypti* (L.) (Diptera: Culicidae) and *Ae. albopictus* (Skuse) (Diptera: Culicidae), respectively), and the presence of a probable salivary gland barrier in *Ae. albopictus* to Zika virus. Finally, we calculated vectorial capacity for both species and found that *Ae. albopictus* had a slightly lower vectorial capacity when compared with *Ae. aegypti*. Second Language Abstract: La rápida expansión del virus Zika, poco después de las epidemias de chikungunya, rebaso la infraestructura de salud pública en muchos países y sorprendió a muchos en la comunidad científica. Notablemente, *Aedes aegypti* y *Aedes albopictus* transmiten estos patógenos además del virus del dengue. En este estudio se expusieron con tres cepas americanas de virus Zika a grupos de *Aedes aegypti* y *Aedes albopictus* de generación reciente. Encontramos altos porcentajes de infección y diseminación en ambas especies (95%). Se reporta, la transmisión viral en ambas especies (74 y 33%, para *Aedes aegypti* and *Aedes albopictus*, respectivamente) y una probable barrera a nivel de glándulas salivarías. Finalmente, calculamos la capacidad vectorial para ambas especies.

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Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Keywords

Aedes aegypti; *Aedes albopictus*; Zika virus; vector competence; vectorial capacity

The rapid expansion of Zika virus (ZIKV) following the recent outbreaks of Chikungunya virus (CHIKV; Staples et al. 2009, Staples and Fischer 2014), overwhelmed the public health infrastructure in many countries (Fauci and Morens 2016, Gulland 2016) and alarmed many in the scientific community (Duffy et al. 2009, Cao-Lormeau et al. 2014, Tognarelli et al. 2016). These pathogens, together with dengue virus (DENV), are primarily transmitted by *Aedes aegypti* (Siler et al. 1926, Marchette et al. 1969) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) mosquitoes (Pagès et al. 2009, Grard et al. 2014).

A mosquito species may be considered vector competent when the species' females transmit the pathogen from one vertebrate to another during blood feeding (World Health Organization 1961). Individually, a mosquito is considered infectious when a pathogen infects its body, disseminates to the salivary glands, and is transmitted to another host. However, barriers within the mosquito can interfere or completely block infection, dissemination, and transmission.

For example, the observed vector competence of *Ae. aegypti* populations can vary with multiple strains of DENV (Dickson et al. 2014) and mosquito collection site. Variation in vector competence, for a given viral strain and a distinct mosquito population, has been observed at worldwide (Tabachnick et al. 1985), regionally (Bennett et al. 2002, Lozano-Fuentes et al. 2009), and at city-scale levels (Gonçalves et al. 2014). Little is known about ZIKV interactions within *Ae. albopictus* (Vanlandingham et al. 2016), but variation has been shown in infection, dissemination, and transmission rates similar to other arboviruses (Li et al. 2012, Wong et al. 2013, Diagne et al. 2015, Aliota et al. 2016, Azar et al. 2017, Ciota et al. 2017, Liu et al. 2017, Roundy et al. 2017).

Recent laboratory experiments demonstrated the extent of *Ae. albopictus* vector competence with variable results (form poorly competent to highly competent) and further incriminated it as a potential vector of currently circulating ZIKV strains (Wong et al. 2013, Chouin-Carneiro et al. 2016, Jupille et al. 2016, Ciota et al. 2017, Liu et al. 2017). The studies demonstrate the inherent variation of vector competence that is observed between various populations of mosquitoes. Specifically, it highlights the need for caution when interpreting the vector status of a species when making local predictions and the need for additional studies targeting local vector populations for evaluation of vector competence.

The essential criteria to consider a species a vector is the ability to transmit the virus under field conditions. Recent field studies suggest that *Ae. albopictus* could be involved in field transmission (Grard et al. 2014, Huerta et al. 2017). In Gabon, testing field-collected mosquitoes using reverse transcription polymerase chain reaction (RT-PCR), researchers concluded that *Ae. albopictus* was the primary vector of a previously undetected ZIKV outbreak (Grard et al. 2014). Huerta et al. 2017 found ZIKV RT-PCR positive mosquito pools of field-collected *Ae. albopictus* and *Ae. aegypti* in Mexico before symptomatic cases occurred, suggesting the probable participation of *Ae. albopictus* in ZIKV outbreaks.

Given its increasing dispersion into temperate regions of the world and its ability to reach latitudes further north than *Ae. aegypti* in China (Wu et al. 2011), Europe (Kraemer et al. 2015, Cunze et al. 2016), and United States (Kraemer et al. 2015; Hahn et al. 2016, 2017), *Ae. albopictus* is of particular interest as a potential vector. We present transmission rate data for field-collected *Ae. albopictus* and *Ae. aegypti* from the Americas challenged with three American strains of ZIKV (Honduras [HON], Miami [MIA], and Puerto Rico [PR]), under laboratory conditions. We also used a mosquito strain (*Ae. aegypti* from Poza Rica, Mexico) with previously reported transmission data (Weger-Lucarelli et al. 2016) as a control.

Material and Methods

Mosquitoes

The mosquitoes used in this study originated from four locations (Fig. 1) and were collected August–September 2016, except for the Poza Rica *Ae. aegypti* which has been in colony since August 2012. The Mississippi *Ae. albopictus* population originated from 8 collection sites from several counties in Mississippi (Hinds, Jasper, Lauderdale, Newton, Smith, Warren, and Yazoo). The North Carolina *Ae. albopictus* population consisted of specimens from four collection sites on the Western Carolina University Campus in Jackson County. The Chiapas *Ae. aegypti* population was collected from four settlements (Tapachula, Escuintla, Pijijiapan, Huixtla) in Chiapas, Mexico. The Poza Rica *Ae. aegypti* colony was established from six collection sites within the city of Poza Rica, Veracruz, Mexico.

Mosquito eggs were hatched with deionized water. Larvae were maintained on a diet of liver powder at a constant temperature of 28°C. Adult mosquitoes were provided a 10% sugar solution ad libitum and kept at a constant temperature of 28°C in a 16:8 (L:D) h cycle with high relative humidity (>75%).

The *Ae. albopictus* mosquitoes from Mississippi and North Carolina were generation F1 or F3, Chiapas *Ae. aegypti* was F2, and Poza Rica was from an unknown advanced generation.

Virus

We used three American strains of ZIKV in the challenges: PR (GenBank: KU501215), HON (GenBank: KX694534), and a novel isolate from MIA confirmed via RT-PCR and sequencing (GenBank: MF988743). The PR strain was isolated from the serum of a febrile traveler returning to the continental United States from PR in 2015. The HON strain originated from the human placenta of a patient who had traveled to HON in 2015 and had one passage in C636 cells and two passages in Vero cells. The MIA strain originated from an *Ae. aegypti* mosquito pool and was utilized in this study after two passages in Vero cells (Mutebi et al. 2018).

Mosquito Exposure

In each challenge (virus strain/mosquito collection combination) at least 30 six to seven-day-old females were engorged on a bloodmeal after being sugar-starved for 24 h.

The infectious stock bloodmeal was prepared with 9 ml of defibrinated calf blood, five ml of fetal bovine serum, 1 ml of the concentrated virus, and to promote feeding, ATP (Pfaltz &

Bauer, Connecticut) at a final concentration of 1 μM. Three milliliters of the bloodmeal stock were added to collagen covered membrane Hemotek feeders (Discovery Workshops, Accrington, United Kingdom), and offered to the mosquitoes for 45 min. The blood meals contained $10^{7.5}$, $10^{6.5}$, and $10^{6.0}$ plaque-forming units per ml (PFU/ml) of PR, HON, and MIA, respectively. After feeding, mosquitoes were anesthetized by brief exposure to −18°C and non-engorged mosquitoes discarded. Engorged females were kept in ~500 ml cartons at 28°C in a high relative humidity environment with 10% sugar solution ad libitum.

Assessment of Infection, Dissemination and Transmission Rate, and Viral Titration

At 14 days post-infection (dpi) mosquitoes were anesthetized with triethylamine (Flynap; Carolina Biological Supply Company, Burlington, NC), had their wings and legs removed, and their proboscis inserted into capillary tubes containing immersion oil (Cargille Laboratories, Cedar Grove, NJ) to collect saliva. Expectoration was conducted for each mosquito for at least 45 min, and at most 90 min for mosquitoes in larger groups.

The legs and wings from each mosquito were placed in a single 2-ml tube (Axygen Scientific) with 500 μl of DMEM media (Dulbecco's Modified Eagle Medium, 2% Fetal Bovine Serum, 10% Penicillin/Streptomycin, 0.4% Amphotericin B) with a copper coated steel BB pellet. The bodies from each specimen were placed in separately labeled tubes with 500 μl of 2% DMEM. All mosquito samples were triturated using an MM300 TissueLyser (Retsch, Newton, PA) for 4 min at 25 cycles/s, and subsequently centrifuged for 4 min at 8,000 revolutions/min (5009 × *g*) at 4°C. Capillary tubes with saliva expectorates were placed directly into a two ml tube containing 400 μl of 2% DMEM media and centrifuged as described above. To detect ZIKV RNA, real-time RT-PCR was performed on all samples using previously reported protocol and primers (Lanciotti et al. 2008, Burkhalter and Savage 2017), on a CFX96 Touch system (Bio-Rad Laboratories). Samples with a cycle threshold (i.e., the number of cycles required for the fluorescent signal to cross the threshold) of <38 were scored as positive.

We titrated RT-PCR positive saliva samples by plaque assay as previously described (Beaty et al. 1989). After a 60-min incubation at 37°C, the plates were overlayed and then incubated for 48 h before adding a second overlay that contained neutral red. Plaques were counted daily from 3 to 7 dpi. Samples that produced 1–100 plaques were used to estimate saliva viral titer.

We equated the proportion of positive bodies to the infection rate. The proportion of positive legs and wings are reported as the dissemination rate indicating that the virus escaped the midgut and disseminated in the mosquito. Virus-positive saliva demonstrated that the virus infected the salivary glands and was expelled into the capillary tube, and is used as the transmission rate.

Estimating the Infection, Dissemination and Transmission in Mosquito Populations

To determine the rates of infection, dissemination, and transmission in the mosquito populations, following an approach analogous to the 'Exact Binomial test' (Sokal and Rohlf 1995), we assumed that the number of positive tissues followed a Binomial distribution, $x \sim \text{Binomial}(\theta, n)$. Where x was the number of positive samples, n was the total number of

samples analyzed, and θ was the unknown rate. Rates were multiplied by 100 to express them as percentages. We opted for Bayesian inference to estimate the θs instead of Frequency or Likelihood inference.

To compare between rate estimates we used 95% high-density intervals (HDI), which are analogous to confidence intervals (CIs), describing the region that contains the estimate with a 95% probability. Similarly to CIs, if HDIs do not overlap, the compared estimates are considered statistically different with a 95% probability. This methodology supersedes null hypothesis testing (Kruschke 2014, Wasserstein and Lazar 2016). The θs and the 95% HDIs were estimated mathematically, using modified R scripts (R Core Team 2014) of Kruschke 2011. Further technical details can be found in the Supp. Material; the data is presented in Supp. S1; scripts will be provided upon request.

Estimating the Mean Saliva Titers

The mean number of plaque forming units per ml (PFU/ml) per species was estimated using a repeated measurements model (Sokal and Rohlf 1995). The model estimates the mean for each individual saliva, and based on those means, it then estimates the mean for the mosquito species. The groups' means were compared using HDIs in a similar fashion to CIs. Further technical details about the model are presented in Supp. S1; scripts and data will be provided upon request.

Vectorial Capacity

We used our vector competence results in conjunction with previously reported ecological data to estimate the theoretical vectorial capacity of each species. To avoid presenting many comparisons, we selected viral challenges with the highest transmission rates. Vectorial capacity (V) describes the ability of a mosquito population to spread a pathogen among hosts. It takes into account the mosquito, the pathogen, and the host, in this case, people. More narrowly, V is the number of potentially infective bites that will be delivered by all vectors feeding on a single host in a day (Fine 1981). We calculated V using formula 13 from Black and Moore 2004,

$$V = \frac{ma^2 p^n b}{-\ln p};$$

where m = mosquito density relative to the host (i.e., the mosquito/person ratio); a = probability a mosquito feeds on a host in a day (host preference index multiplied by the feeding frequency on the host); b = proportion of mosquitoes ingesting an infective meal that become infectious (transmission rate); p = probability the mosquito will survive one day; n = extrinsic incubation period.

For the m parameter, we used the estimated number of *Ae. aegypti* females per person in a neighborhood located in Brazil for two relevant periods: during a dengue epidemic, with a density of 4,100 mosquitoes per 2,350 people (m = 1.75), and before the epidemic, with a density of 700 mosquitoes per 2,350 people (m = 0.30; Villela et al. 2015).

For *a*, we selected different values for each species given their dissimilar feeding habits. Being mostly anthropophagic, we chose $a = 0.23$ for *Ae. aegypti*. The values derived from *Ae. aegypti* feeding almost exclusively on people with a 0.90 host preference (Wong et al. 2011) and a 3-d gonotrophic cycle ((McClelland and Conway 1971, Jansen et al. 2015) for a daily feeding frequency of 0.33. Feeding primarily on mammals, *Ae. albopictus* is known to be highly opportunistic (Savage et al. 1993). For *Ae. albopictus*, using the same feeding frequency as *Ae. aegypti*, we selected two values, $a = 0.23$ and $a = 0.13$, corresponding to host preferences of 0.90 (Ponlawat and Harrington 2005, Kamgang et al. 2012) and 0.50 (Savage et al. 1993, Richards et al. 2006, Faraji et al. 2014). To establish a point of comparison, for *b*, we selected the viral challenges with the highest transmission rates obtained in this study and their 95% HDIs. For *P*, we selected 0.85 for both species (Maciel-de-Freitas et al. 2006, David et al. 2009, Jansen et al. 2015). Finally, for the *n* parameter we used 7 days for both species (Chan and Johansson 2012).

Results

Infection, Dissemination and Transmission Rates

The infection rate varied among mosquito collections, but primarily across virus strains; very likely because of differences in bloodmeal titer. Examining together the infection and dissemination rates, *Ae. albopictus* had relatively higher rates than *Ae. aegypti* when exposed to lower titer blood meals (HON and MIA); however, the increased infection and dissemination rates did not translate into higher transmission rates for *Ae. albopictus*. Additionally, all infection and dissemination rates in the HON and MIA challenges are statistically lower than the PR challenges (i.e., non-overlapping HDIs), excepting Poza Rica. Furthermore, it is noteworthy that the dissemination and infection rates were not statistically different in all 12 challenges (Fig. 2, Supp. S1)

The transmission rates in *Ae. aegypti* varied between 6 and 74% (Fig. 2). Interestingly, the Chiapas *Ae. aegypti* challenges showed the lowest and the highest transmission rates in *Ae. aegypti* when exposed to MIA and PR respectively. The MIA/Chiapas challenge was not statistically different from zero (i.e., the HDI contains a transmission rate of zero). The *Ae. albopictus* challenges also showed variation in the transmission rate, although somewhat lower (7–33%). The *Ae. albopictus*/PR challenges showed the highest transmission rate (25–33%), followed by HON (5–14%) and MIA (3–7%); a likely result of the differences in bloodmeal viral titers. Three out of six *Ae. albopictus* challenges had a low transmission rate and were not statistically different from zero.

Generally, the *Ae. albopictus* challenges showed statistical differences (probability ≥ 95%) between the transmission and the infection rates, excepting MIA/North Carolina, while *Ae. aegypti* populations did not (Fig. 2). Additionally, the highest *Ae. albopictus* transmission rate (33%, 95% HDI [14%, 53%]) is statistically different from the highest *Ae. aegypti* rate (74% [54%, 92%]). This statistical difference in transmission rates between the species was only observed in the PR challenges whereas the other strains showed no statistical differences in transmission rates for each species (Fig. 2.)

Saliva Virus Titer

The saliva virus titer results are presented in Fig. 3 and Supp. S1, Fig. 2. Only 19 out of the 31 RT-PCR positive saliva samples from all *Ae. aegypti* challenges generated plaques, however, each positive saliva was measured three to five times. The saliva samples with viable virus ($n = 19$) had a mean titer (μ_G) of $10^{2.7}$ ($10^{2.4}$, $10^{3.0}$) PFU/ml (Fig. 3). The developed model fitted the data well; only three observations fall outside the region drawn by SD times two (Fig. 3). From all 12 challenges, only PR/Chiapas had a sample size large enough to allow a separate analysis ($n = 10$), this challenge had a saliva titer of $10^{2.6}$ ($10^{2.2}$, $10^{2.9}$). The precision of the mean and SD in the other challenges was small due to small sample sizes (PR/Poza Rica = 1, HON/Chiapas = 3, HON/Poza Rica = 4, MIA/Poza Rica = 1, MIA/Chiapas = 0); therefore, the estimations are not shown.

In the *Ae. albopictus* challenges, 7/12 RT-PCR positive saliva samples resulted in viable virus replication detected by plaque assay, again, each positive saliva was measured three to five times. The saliva had a mean titer of $10^{2.1}$ ($10^{1.8}$, $10^{2.3}$), but the model does a relatively poorer job describing the data for *Ae. albopictus* (Fig. 3). The most probable mean is located in a small range of values, but the SD is relatively narrow and does not capture a few observations. Only PR challenges generated plaques; three samples came from the Mississippi population and four from the North Carolina population. Bearing in mind the fit of the *Ae. albopictus* model, *Ae. aegypti* had a significantly larger saliva virus titers ($10^{2.7}$ [$10^{2.4}$, $10^{3.0}$]) than *Ae. albopictus* ($10^{2.1}$ [$10^{1.8}$, $10^{2.3}$]).

Vectorial Capacity

We calculated V for each virus strain by selecting from each mosquito species the challenge with the highest transmission rate and its 95% probability interval. Using dengue as a reference (Villela et al. 2015), we developed scenarios where the female mosquito density would represent two critical epidemiological periods: the epidemic density (1.8 mosquitoes per person) and the non-epidemic density (0.3 mosquitoes per person). Values for the other V parameters were described above.

With a most probable transmission rate of 74% (when exposed to PR) and with an epidemic density, *Ae. aegypti* had the highest V (=0.22; Fig. 4). The *Ae. aegypti* differences in V are considerable, especially when comparing non-epidemic density and challenges with lower bloodmeal titers (HON and MIA).

Regardless of host preference level, *Ae. albopictus* had a lower V than *Ae. aegypti* with all viral strains. For example, during the epidemic period and with a host preference index of 0.90, $V = 0.10$, 0.04, and 0.02 for PR, HON, and MIA, respectively. The reductions are particularly noticeable when compared to the largest possible V (0.35; Fig. 4, top dashed line), obtained with a transmission rate of 100% and a host preference index of 0.90.

Discussion

The comparisons between different vector competence experiments should be done keeping in mind that there is considerable variation among studies. Some of the sources of variation are virus strains, cell culture methodology (and cell type), and virus analysis methodology

which can affect the results of a vector competence experiment. We discuss other studies as a point of reference while considering the caveats.

Our saliva analysis showed that the most probable mean number of ZIKV plaque forming units per ml was $10^{2.7}$ and $10^{2.1}$ for *Ae. aegypti* and *Ae. Albopictus*, respectively. Dubrulle et al. 2009 found similar values for *Ae. albopictus* ($10^{3.3}$) and *Ae. aegypti* ($10^{2.5}$) when exposed to the 6.21 strain of CHIKV (at titer $10^{7.5}$ PFU/ml). Poole-Smith et al. 2015 challenged *Ae. aegypti* with the four serotypes of DENV, 10^5–10^6 PFU/ml, and observed that the highest saliva titers for DENV ($10^{0.3}$–$10^{2.9}$) were similar to our results for ZIKV.

We obtained results similar to those found by Weger-Lucarelli et al. 2016 when comparing the Poza Rica *Ae. aegypti* when exposed to $10^{7.2}$ PFU/ml of the ZIKV PR strain. Chiapas *Ae. aegypti* had a statistically higher transmission rate with PR than the Poza Rica specimens. Chiapas' transmission rate was also higher than those found by Roundy et al. 2017 using a $10^{6.0}$ focus forming unit/ml of a different American ZIKV strain (GenBank: KX247632.1) from Mexico and *Ae. aegypti* from El Salvador.

We observed a higher transmission rate in our *Ae. albopictus* challenges with PR than those found by Chouin-Carneiro et al. 2016 using a Vero Beach colony. However, the rates in the MIA and HON challenges were similar to those observed by Chouin-Carneiro et al. 2016 and Jupille et al. 2016 with mosquitoes from Florida and France, respectively. Both, Chouin-Carneiro et al. 2016 and Jupille et al. 2016, used a New Caledonia ZIKV strain (NC-2014–5132) with bloodmeal titers of ~$10^{6.8}$ PFU/ml. Our *Ae. albopictus* data contrasts sharply with the transmission rate of 100% obtained using Singapore mosquitoes and ~$10^{7.3}$ PFU/ml of Ugandan MR766 ZIKV (Wong et al. 2013).

The transmission rate is mainly dependent on the infection and dissemination rates. However, it is noteworthy that in our study the infection and dissemination rates were not statistically different, indicating that a midgut escape barrier was probably not present in either species.

Chouin-Carneiro et al. 2016 showed reductions in the dissemination rate at 14 DPI, while in all our challenges we observed that the infection and dissemination rates were not statistically different in both species at 14 DPI. Interestingly, when exposed to low titer blood meals (HON and MIA), *Ae. albopictus* had relatively higher infection and dissemination rates (average = 39%) than *Ae. aegypti* (average = 24%). Nevertheless, the increase did not translate to higher transmission for *Ae. albopictus* with an average transmission rate of 7%; *Ae. aegypti* had an average transmission rate of 14%. Ciota et al. 2017 showed similar results when exposing *Ae. albopictus* and *Ae. aegypti* to ~$10^{6.0}$ HON strain (KX906952).

The statistical differences between infection and transmission rates in five of the six *Ae. albopictus* challenges suggest that a salivary gland barrier is likely present in *Ae. albopictus* but not in *Ae. aegypti*. This indicates that the main difference between *Ae. aegypti* and *Ae. albopictus* in their interaction with ZIKV is the presence of a probable salivary gland barrier in multiple populations of *Ae. albopictus*, which was also observed by others (Azar et al.

2017, Ciota et al. 2017). Further studies with *Ae. albopictus* would be required to assess if the probable salivary barrier is an infection barrier or an escape barrier.

Additional differences between the *Ae. aegypti* and *Ae. albopictus* can be observed in their V. The dissimilarities are a direct result of the reduction in transmission rate; *Ae. albopictus* presented lower V values with the same host preference as *Ae. aegypti*. The lower V in *Ae. albopictus*, together with the lower saliva titer, points to *Ae. aegypti* being the primary driver of ZIKV epidemics in areas where these species are sympatric but do not preclude the transmission of ZIKV by *Ae. albopictus* in regions where only the latter is present.

Interestingly, the differences in V between the two species are considerably reduced in trials with lower blood meals titers. These reductions followed decreases in the transmission rate. Also, despite the relatively small transmission rates in *Ae. albopictus*, only MIA had a V with an interval that included zero during an epidemic period, indicating that, in high densities, *Ae. albopictus* could potentially infect people with PR, and HON, at the observed viral titers, regardless of host preference.

The relatively small V values (*Ae. aegypti* 0.22, *Ae. albopictus* 0.10; obtained selecting the PR strain and the epidemic density) seem inconsistent with the ZIKV outbreaks observed throughout the world. However, this situation—outbreaks driven by vectors with low vectorial capacity—could be emphasizing the importance the susceptible to immune host ratio had in the speed and spread of the ZIKV. Another possibility that could explain the outbreaks in low V scenarios is that vector competence experiments do not capture the host-adaptation process, where the best adapted viral strain is selected (Deardorff et al. 2011). However, even with a transmission rate of 1.0, the values of V would remain relatively low (0.35). Another consideration is that *Ae. aegpti* is known to feed multiple times before completing a gonothrophic cycle (Pant and Yasuno 1973, Scott et al. 1993). Based on field observations (Pant and Yasuno 1973), we created a scenario where 20% of the mosquitoes fed twice per gonothrophic cycle. Keeping a 3-d gonotrophic cycle and doubling the number of bites for 20% of the mosquitos, would increase the daily bitting rate to 0.4 (daily feeding frequency = ((80% * 0.33) + (20% * 0.66))/100). As expected under this scenario, the V for *Ae. aegypti* exposed to PR was more than double (0.54).

Another point to consider is that daily survival (P) has a substantial impact on V. For example, selecting the highest most probable transmission rates, the epidemic density, and increasing P from 0.85 to 0.90 (other parameters remaining equal), V is increased two-fold, from 0.22 to 0.51, and from 0.10 to 0.23 for *Ae. aegypti* and *Ae. albopictus* respectively. Another possible scenario could be found in areas where *Ae. aegypti* has a slightly lower P than *Ae. albopictus*. A decrease in P in *Ae. aegypti* from 0.85 to 0.75 (other parameters equal), dramatically reduces V four-fold (from 0.22 to 0.05), this lower estimate would be half the V obtained for *Ae. albopictus* ($b = 0.53$, $p = 0.85$, and a host preference index = 0.9). The importance of the differences in daily survival between species becomes evident when we consider that *Ae. albopictus* is better adapted to colder weather than *Ae. aegypti* and therefore it is likely to have a higher daily survival (Brady et al. 2016). In the United States alone, the observed range of *Ae. albopictus* is larger by 14 states than *Ae. aegypti*

(Hahn et al. 2016, 2017). In temperate areas that are not occupied by *Ae. aegypti*, *Ae. albopictus* is positioned to put people at risk of infection with ZIKV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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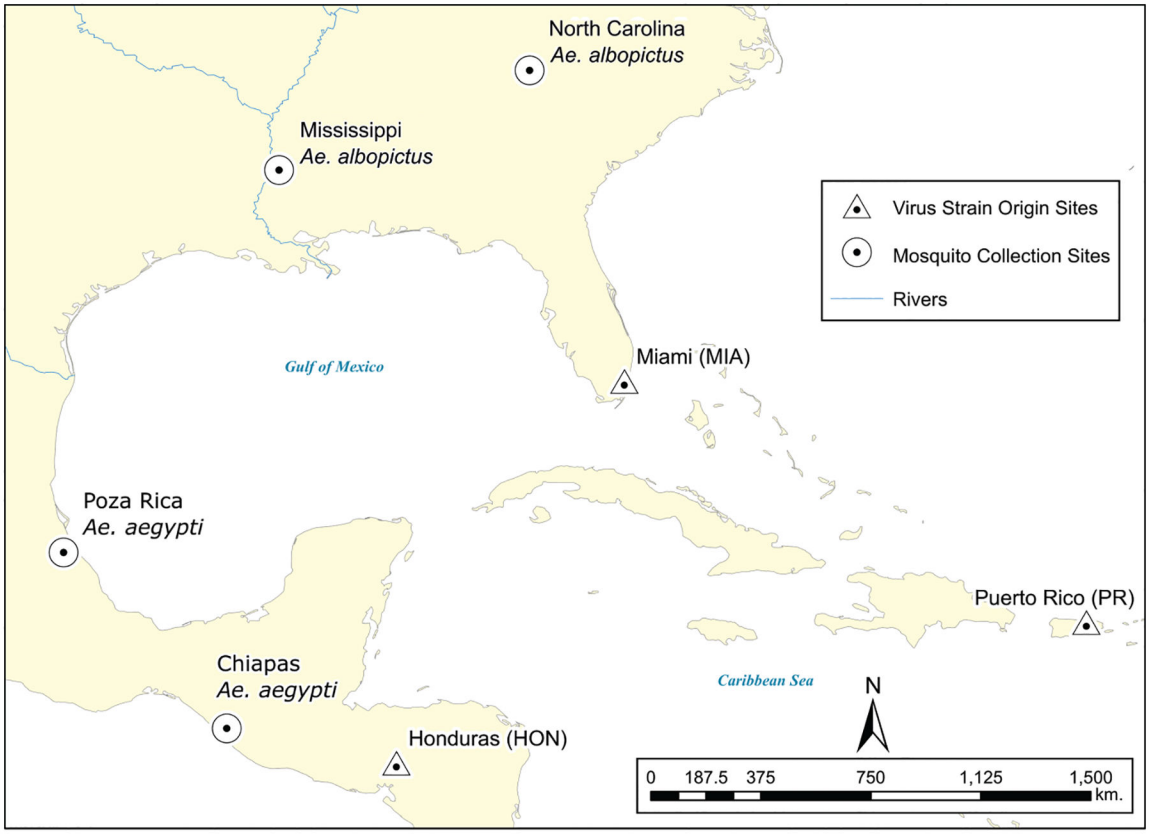

Fig. 1.
Mosquito collection sites and ZIKV strains place of origin.

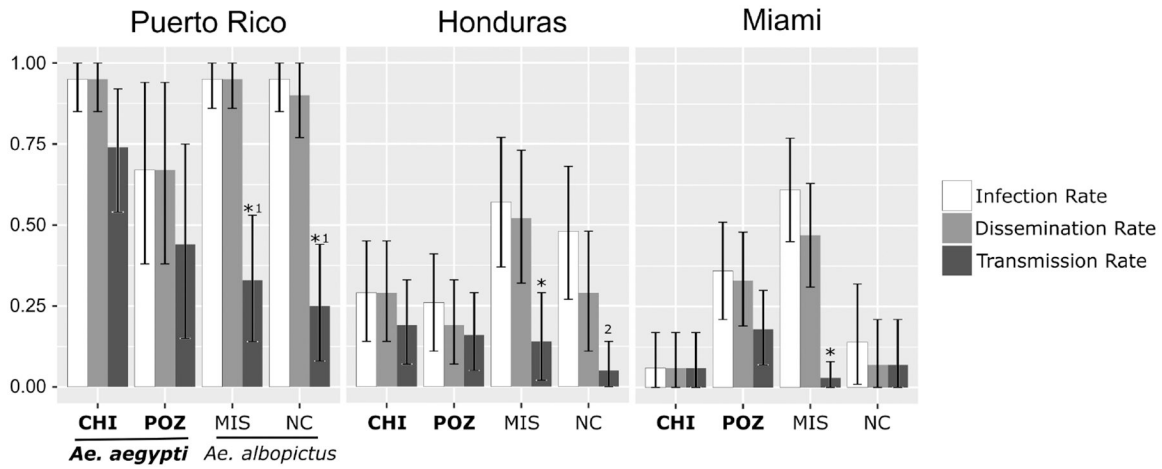

Fig. 2.

Infection, dissemination, and transmission rate for two groups of *Ae. aegypti* (Chiapas [CHI] and Poza Rica [POZ]) and two groups of *Ae. albopictus* (Mississippi [MIS] and North Carolina [NC]), challenged with three strains of ZIKV (Puerto Rico, Honduras, and Miami). The top of the bar indicates the rate and the error bars indicate the 95% HDI. *A statistical difference (probability ≥ 95%) between the transmission rate and both infection and dissemination rates. 1PR challenge transmission rate statistically different (probability ≥ 95%) from the PR/Chiapas challenge transmission rate. 2A statistical difference (probability ≥ 95%) between the transmission and the infection rate.

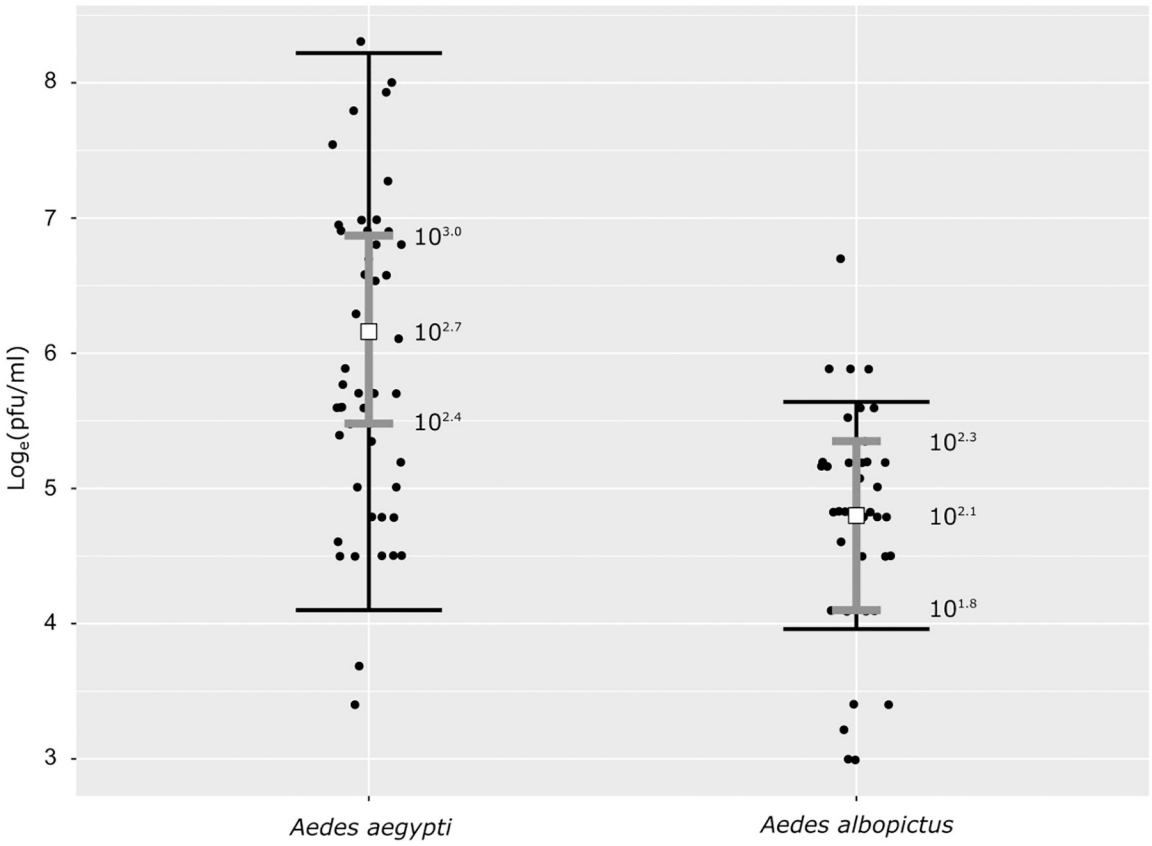

Fig. 3.

Mean PFU/ml (μ_G) and SD (σ_G) for RT-PCR positive saliva from *Ae. aegypti* and *Ae. albopictus* (black dots) in base *e* logarithm. The center white square denotes the μ_G for each species. The narrow (gray) error bars show the HDIs for the estimated means. The wider error bars (black) represent two times the σ_G (or 95.4% area under the curve of a normal distribution). The values next to the mean error bar are the back-transformed (from $\log_e$ to $\log_{10}$) values of the mean and its 95% HDIs.

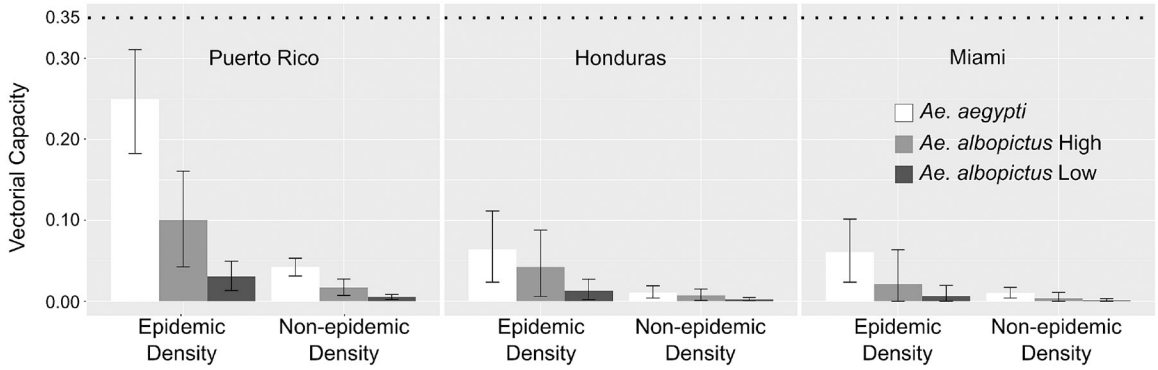

Fig. 4.

Vectorial capacity of American *Aedes aegypti* and *Aedes albopictus* to three strains of American ZIKV. '*Aedes albopictus* High' corresponds to *Ae. albopictus* having a host preference index of 0.90 while '*Aedes albopictus* Low' corresponds to a preference index of 0.50. The top dotted line represents a vectorial capacity with a transmission rate of 1.00 and a host preference index of 0.90 (see text for other parameters). The epidemic density represents the number of female mosquitoes per person during a dengue epidemic in Brazil (Villela et al. 2015). The non-epidemic represents the observed density before the dengue epidemic.