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Surveillance of *Aedes aegypti* indoors and outdoors using Autocidal Gravid Ovitraps in South Texas during local transmission of Zika virus, 2016 to 2018

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ABSTRACT

The yellow fever mosquito, *Aedes aegypti*, has facilitated the re-emergence of dengue virus (DENV) and emergence of chikungunya virus (CHIKV) and Zika virus (ZIKV) in the Americas and the Caribbean. The recent transmission of these arboviruses in the continental United States has been limited, to date, to South Florida and South Texas despite *Ae. aegypti* occurring over a much larger geographical region within the country. The main goal of our study was to provide the first long term longitudinal study of *Ae. aegypti* and enhance the knowledge about the indoor and outdoor relative abundance of *Ae. aegypti* as a proxy for mosquito-human contact in South Texas, a region of the United States that is at high risk for mosquito-borne virus transmission. Here, the relative abundance of indoors and outdoors mosquitoes of households in eight different communities was described. Surveillance was done weekly from September 2016 to April 2018 using the CDC Autocidal Gravid Ovitraps in low- and middle-income communities. A total of 69 houses were included in this survey among which 36 were in the low-income communities (n = 11 for Donna, n = 15 for Progresso, n = 5 for Mesquite, n = 5 for Chapa) and 33 in middle-income communities (n = 9 for La Feria, n = 8 for Weslaco, n = 11 for McAllen, and n = 5 for Rio Rico). Overall, *Ae. aegypti* was the dominant species (59.2% of collections, n = 7255) followed by *Culex* spp. mosquitoes (27.3% of collections, n = 3350). Furthermore, we demonstrated for *Ae. aegypti* that 1) outdoor relative abundance was higher compared to indoor relative abundance, 2) low-income communities were associated with an increase in mosquito relative abundance indoors when compared to middle-income communities, 3) no difference was observed in the number of mosquitoes collected outdoors between low-income and middle-income communities, and 4) warmer months were positively correlated with outdoor relative abundance whereas no seasonality was observed in the relative abundance of mosquitoes indoors. Additionally, *Ae. aegypti* mosquitoes collected in South Texas were tested using a specific ZIKV/CHIKV multiplex real-time PCR assay, however, none of the mosquitoes tested positive. Our data highlights the occurrence of mosquitoes indoors in the continental United States and that adults are collected nearly every week of the calendar year. These mosquito data, obtained concurrently with local ZIKV transmission of 10 locally acquired cases in nearby communities, represent a baseline for future studies in the Lower Rio Grande Valley (LRGV) including vector control interventions relying on the oviposition behavior to reduce mosquito populations and pathogen transmission.

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1. Introduction

Mosquito-borne viruses, driven principally by *Aedes aegypti*, continue to emerge and re-emerge globally (Kean et al., 2015). After dengue virus (DENV) arrived to the Americas and caused major and widespread epidemics, vector control successfully limited the distribution of the vector and disease (Soper, 1963, 1965). However, globalization and the return of the vector to cities in tropical and subtropical regions of the world resulted in the global re-emergence of DENV (Brady et al., 2014). In 2017 alone, an estimated 468,000 human cases were reported throughout the Americas (PAHO, 2018). Similarly, chikungunya virus (CHIKV) made the jump to the Western Hemisphere in 2013, spreading through the Caribbean and quickly to much of the Americas, resulting in 122,951 confirmed cases (PAHO, 2017). Zika virus (ZIKV) followed a similar pattern of emergence. In 2014, the virus appeared in northeastern Brazil and spread throughout much of the Americas and the Caribbean, emerging for the first time in regions with endemic DENV and CHIKV (Roth et al., 2014).

Aedes aegypti is the major vector of ZIKV, CHIKV and DENV in the New World (Chouin-Carneiro et al., 2016; Lounibos and Kramer, 2016; Lourenco-de-Oliveira et al., 2004; Roundy et al., 2017), and the persistence and spread of this mosquito facilitates arboviral transmission (Powell and Tabachnick, 2013). The relative abundance of *Ae. aegypti* is increasing with population growth, urbanization, poverty, and a throw-away society of plastic and other containers that contributes to the available larval habitat (Gubler, 1989, 1996; Weaver and Barrett, 2004; Weaver and Reisen, 2010). This mosquito is highly anthropophilic and endophilic, which increases mosquito-human contact rates and amplifies arboviral transmission (Carrington and Simmons, 2014).

In the continental United States (U.S.), local mosquito-borne transmission of DENV, CHIKV, and ZIKV has been restricted to South Florida (DENV and ZIKV) and South Texas (DENV, CHIKV and ZIKV) despite the presence of *Ae. aegypti* in 26 out of the 50 U.S. states (Hahn et al., 2017; Johnson et al., 2017). Even in areas where *Ae. aegypti* populations occur within the U.S., (Hahn et al., 2016), data on their relative abundance and seasonality are often missing. *Aedes aegypti* indoor feeding rates are assumed to be very low, decreasing the probability of mosquito-human contact and thus lowering the potential for viral persistence in local mosquito populations (Brunkard et al., 2007; LaDeau et al., 2013; Ramos et al., 2008). However, the degree to which mosquitoes feed on humans inside homes in the continental U.S. is poorly understood, principally due to the difficulty in sampling these populations in the sensitive environment of an individual's home.

There are a variety of tools that are currently used for mosquito vector surveillance. Adult mosquito abundance has been identified as a better entomological index for the risk of arbovirus transmission as opposed to egg, larval or pupal indices (Sivagnaname and Gunasekaran, 2012). Accordingly, for this study we use the Center for Disease Control and Prevention (CDC) Autocidal Gravid Ovitrap (AGO), which provides a measure of adult relative abundance that correlates to other adult traps such as the BG Sentinel trap (Barrera et al., 2014a). Using this surveillance tool, prior studies have identified that a catch rate of less than three adult females *Ae. aegypti* per AGO per week is able to reduce the risk of CHIKV incidence (Barrera et al., 2017; Lorenzi et al., 2016).

With the repetitive introduction of arboviruses in South Texas, there is a need to better understand *Ae. aegypti* abundance and distribution in residential areas as well as the drivers responsible for the variation in abundance. Given that little attention to sampling mosquitoes indoors occurs in the continental U.S., we tested the hypothesis that AGO traps would catch mosquitoes from the outdoor environment but not inside human homes. To achieve this, we deployed a standardized surveillance system with the use of AGO indoors and outdoors in low- and middle-income communities in South Texas to 1) understand the community of mosquitoes attracted to the AGO, 2) determine if income can influence mosquito relative abundance indoor and outdoor and thus learn more about endophily in residential communities and 3) investigate how temperature influences the relative abundance of mosquito populations found in the indoor and outdoor environment. The current study was implemented from September, 2016 to April, 2018 which was concurrent with local mosquito-borne Zika virus transmission in humans in nearby communities.

2. Material and methods

2.1. Study area

The LRGV is located in South Texas, U.S. at the border with Mexico and consists of four counties (Hidalgo, Cameron, Willacy, and Starr) with over 1.3 million people, of which about 90% are Hispanic (2010 Census). The LRGV contains many un-incorporated, rural communities called 'colonias' that lack basic services such as sewage, potable water, or waste management (Carter et al., 1996; Hargrove et al., 2015; Homedes and Ugalde, 2003). Hidalgo and Cameron County are composed of a mixture of incorporated communities and 937 and 196 colonias, respectively (Hidalgo County, Health and Human Services). Because we hypothesize that mosquito abundance is influenced by socio-economic factors, the present study was conducted in eight communities in Hidalgo and Cameron Counties, that were selected, based on income level, number of homes in the community, and degree of isolation from other residential communities.

2.2. Site selection

The 2010 census block groups were used to constrain candidate communities that had a mean household income of $15,000 to $29,999 for low-income or $30,000 to $40,000 for middle-income. There were very few census block groups with mean household incomes below $15,000. Once candidate communities in census block groups were selected within these census block groups, we used Google Earth to identify residential neighborhoods that were more isolated from surrounding residential neighborhoods. Doing this would mitigate confounding effects of immigration of mosquitoes from elsewhere. Finally, the selection of the final eight communities relied on the willingness of the household to participate in this study and on safety of the project personnel. While approaching homes, the first attempt to contact homeowners was during the morning or mid-day. If unsuccessful, we visited later in the afternoon. Finally, if we continued to be unsuccessful, we visited on the weekend. A project flyer (Spanish on one side and English on the other side) explaining the nature of our project and our contact information was left after each unsuccessful attempt.

The four field sites within the low-income census block groups included colonias Balli (n = 11 sentinel homes), Progreso (n = 15), Chapa (n = 5) and Mesquite (n = 5; Fig. 1). The four field sites within middle-income census block groups were the incorporated neighborhoods of La Vista (n = 11), Christian Court (n = 8), the colonias La Bonita (n = 9) and Rio Rico (n = 5) (Table 1; Figure S1). This project was approved by the Texas A&M University Institutional Review Board Permit Number IRB2016-0494D.

2.3. Mosquito sampling

We estimated adult mosquito relative abundance using the CDC AGO traps originally acquired from the CDC Dengue Branch in Puerto Rico during 2016 and from SpringStar Inc. during 2017. Although the recommended baiting dose by the CDC was 30 g of hay, we used one tenth of the suggested dose, 3 g of hay with 10 liters of water for all AGO traps, both outdoor and indoor locations (Figure S2). A lower dose was used because early attempts using the full dose resulted in a strong odor, which was unacceptable for the indoor locations. We acquired hay bales from a local farm and ranch store which was 100% coastal grass; 3 g bundles were prepared ahead of time in the lab using a twist tie. Traps were checked weekly for the entire time of the study (at the

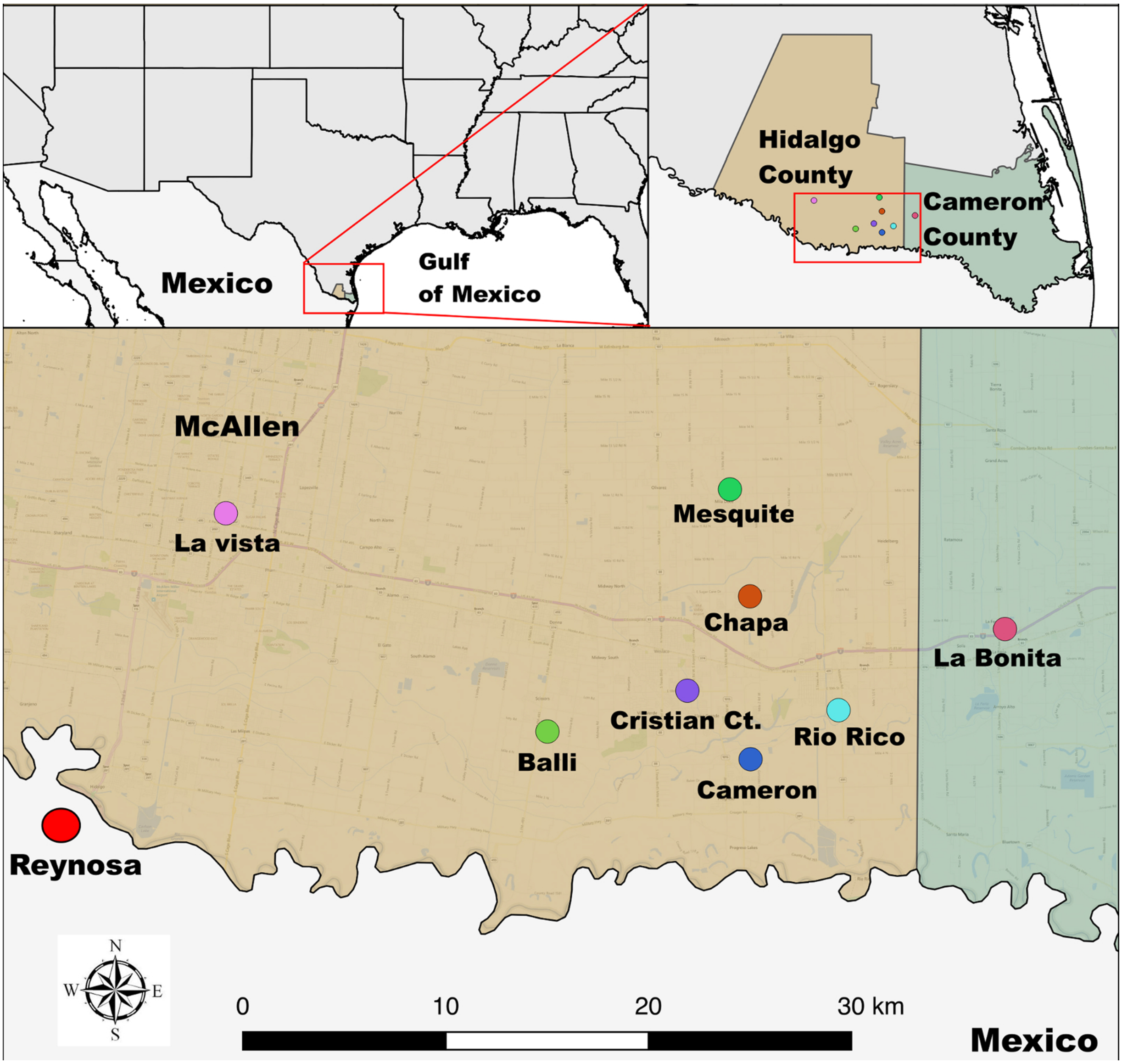

Fig. 1. Map of study area. Eight communities surveyed during this study within the Hidalgo and Cameron Counties in the Lower Rio Grande Valley in South Texas. Surveyed communities appear as different colored markers. The map was developed using QGIS 2.14 with publicly available administrative boundaries.

Table 1
Participation characteristics in eight communities in South Texas involved in the surveillance program using AGOs.

Income	Communities	Participation		
		Total approached[a]	Total visits[b]	Max enrolled (%)[c]
Low-income ($15,000-$29,999)	Balli	33	84	11 (33%)
	Progreso	82	127	15 (18%)
	Chapa	29	59	5 (17%)
	Mesquite	32	40	5 (16%)
Middle-income ($30.000-$40,000)	La Vista	57	252	11 (19%)
	Christian Ct.	32	167	8 (25%)
	Rio Rico	20	87	5 (25%)
	La Bonita	64	139	9 (14%)

[a] Total number of houses approached per community.
[b] Total number of visits per community.
[c] Maximum number of households enrolled per community and percentage of households enrolled per community.

exception of holidays) and mosquitoes were identified on the spot using morphological characteristics. Mosquitoes from each trap were removed from the sticky paper using a teasing needle and pooled per species and sex, and stored at −20 °C to −80 °C until further processing. Trap water and hay bundles were changed weekly and glue board paper was changed as necessary, which was about once every 2 months. The mosquito data collected between September, 2016 and April, 2018 represent base-line mosquito relative abundance with no known municipal mosquito control activities in these eight communities. From September, 2017 to December, 2017, half of these eight communities were randomly identified as 'intervention' communities where we scaled-up the deployment of traps to three AGOs per house outdoors. We did not observe a difference in the number of mosquitoes per AGO per week between intervention and reference communities. Accordingly, all the AGO data at the sentinel homes before, during, and after this intervention are combined for the purpose of this study.

2.4. ZIKV and CHIKV detection using RT-qPCR

Among all the mosquito collected during this study, only the *Ae. aegypti* female were further processed for virus testing. Each *Ae. aegypti* pool was homogenized with a single bead in 600 μL of Hank's buffer solution (Gibco cat# 14,170,112) and centrifuged for 5 min at 18,000 g. RNA was extracted from 100 μL of the supernatant using the MagMax Core kit following the manufacturer's recommendations and tested using a ZIKV and CHIKV multiplex reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) test using the PathID Multiplex one step RT-PCR kit (Lanciotti et al., 2007, 2008). Briefly, 5 μL of RNA was added to a master mix containing 0.3 μM of each probe, 2 μL of each of the four primers, 2.5 μL of enzyme mix and water. Zika PRVABC59 RNA lysate and CHIKV R80422a (India 2006) RNA lysate received from the CDC were used as positive control. Cycling conditions started with the reverse transcription cycle at 48 °C for 10 min, followed by an RT-enzyme inactivation cycle at 95 °C for 10 min and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Samples with Ct values > 38 were considered negative (Lanciotti et al., 2008).

2.5. Statistical analysis

Because of its nature, our study resulted in unbalanced data. For example, only a subset of homes within each community was included for the indoor and outdoor sampling. Additionally, the study relied on the presence of homeowners to gain access to the traps resulting in variation in the time interval between servicing each of the AGO traps. Homes were added and some withdrew at different times during the study (see supplemental Fig. 2), resulting in different numbers of traps in each community. To account for these unbalanced data, we used a generalized linear mixed model (GLMM) framework (implemented in the package of glmmTMB in R) (Bates et al., 2015) with a random error structure to account for variability in the number of samples across trap/homes, communities, and time. Specifically, a nested random effect for homes that occurred within communities and month of sampling was modeled. In addition, the offset function in R was used to account for variation in the length of time each trap was deployed before collecting the captured mosquitoes. Random effects were only included in the model when the variance estimate was greater than zero. Fixed effects consisted of traps placement (indoors, outdoors), socio-economic status (low-, middle-income levels), season (cold; December to February and warm; March to November season), and the interactions between placement and socio-economic status as well as between placement and seasonality. We first tested for the best-fit model of the data using the glmmTMB package in R. Briefly, negative binomial 1, negative binomial 2, Poisson and Gaussian models were tested individually as well as their zero-inflated counterpart to model count data that have an excess of zero values. The negative binomial 2 was the best fit (lowest Akaike's information criterion score). To assess if our best-fit model (negative binomial 2) is able to explain our data well, we performed a model diagnostic using the package DHARMa in R. Because no significant difference was observed between the residuals and the predicted versus observed data, we used the negative binomial 2 model to explore the interaction between "income" and "placement" or "season" and "placement" and to correct for the over dispersion typical of count data.

The variability in the number of *Ae. aegypti* female collected indoor and outdoor in low-income and middle-income communities was examined in the first model. To compare the number of *Ae. aegypti* females collected inside or outside in each community over the span of the study, we ran the GLMM model using fixed variables of trap placement and the income level and the interaction of both placement and income. Each *Ae. aegypti* female mosquito pool collected from the same house on the same day was considered as a trap event and was treated as a pair with an independent indoor trap value and an independent outdoor trap value. The random variable coded every sampling event by the trap ID/ House ID, the community in which the trap belongs and the time each trap was present in the study. The best model was selected by comparing the differential Akaike's information criterion (dAIC) and corresponded to the model including the interaction of placement and income. Confidence intervals of the fixed effects in the best fitted model were obtained using the uniroot function implemented in the package glmmTMB.

To compare the mean number of female *Ae. aegypti* captured indoor versus outdoors in low- and middle-income communities, we estimated marginal means using the "emmeans" package in R and compared them with the "lsmean" R function. If the confidence intervals calculated did not include the value zero, then the difference was considered significant.

To understand the variation in *Ae. aegypti* females collected indoors and outdoors during the cold and the warm seasons, a new model was created using the trap placement, the season and the interaction between the trap placement and the season as fixed variables. For each house a specific trap event was created every week for the indoor and outdoor trap where the number of female *Ae. aegypti* collected was recorded. Similar to the first model, we account for the variability in sampling according to the trap, the community and the time the trap was involved in the study by using the trap ID, community ID (nested) and Month as a random factor. For female *Ae. aegypti,* a zero inflated negative binomial 2 model was used. To compare the mean of female *Ae. aegypti* captured indoors versus outdoors during the cold and the warm seasons, we performed the same post-hoc test as described above.

For the seasonality analysis, mosquito counts were grouped into two groups: cold season representing the months from December to February and the warm season from March to November (Monaghan et al., 2016). The cold season average temperature was 19.2 °C with a minimum average of 10 °C and a maximum average of 25.5 °C. For the warm season, the average temperature was 26.9 °C with a minimum average of 18.3 °C and a maximum average of 30 °C. Weather data were obtained from the McAllen Miller International station. The average weekly temperature each month was calculated by taking the average of the daily temperatures for the week. The information on human ZIKV cases in 2016 and 2017 was provided by county and state health departments and represent the first report of local ZIKV transmission in Texas.

3. Results

3.1. Study participation

In total, 69 homes were surveyed during the study (Table 1), including 11 from Balli (33% of homes approached in the community, n = 33), 15 for Progreso (18%, n = 82), 5 for Chapa (17%, n = 29), 9 for La Bonita (14%, n = 64), 8 for Christian Court (25%, n = 32), 5 for Rio Rico (25%, n = 20), 11 for La Vista (19%, n = 64) and 5 for Mesquite (16%, 32). Over the 386 homes constituting these eight communities, 349 homes were approached over a total number of 955 visits. Of the 349 homes visited within these eight communities, 176 were in low-income communities and 173 in middle-income communities. Overall during the visits, 226 (65%) households did not answer at the time of the visit. Each home was visited at least three times before a decision was taken to categorize them as unavailable. No statistical difference was observed in the percentage of absent homeowners according to income level ($p = 0.181$) in low-income communities (68%) when compared to middle-income communities (61%). Of the 123 homes in which contact with the homeowner was established and the objective of this project was discussed, 69 homes (56%) agreed to participate in the surveillance study by placing an AGO trap inside and outside their home while 54 homes did not consent (44%) (Table 2). When we looked at participation rate by income level, no difference was observed ($p = 0.104$) in low-income communities (64%) versus middle-income communities (49%). Thirty-six of the participating homes (52%) withdrew from the study before its completion (Table 2; Figure S2). Overall the percentage of withdrawal was similar between the low- and middle-income communities ($p = 0.23$) with a 44% withdraw rate in low-income communities and a 60% withdraw rate in middle-income

Table 2
Summary of enrollment history in low-income and middle-income communities in South Texas.

	Home Contact		Among present	Among Consent
	Absent %(n)	Present %(n)	Consent %(n)	Withdraw %(n)
Low-income	68(120)	32(56)	64(36)	44(16)
Middle-income	61(106)	39(67)	49(33)	60(20)

communities. The main reasons for withdrawing were the loss of interest in the study, concern about traps (e.g. smell, danger for children), the high turnover of homeowners, and medical conditions. When we looked more in depth in these different characteristics, we found that whereas no differences were observed between low- and middle-income in terms of withdraw due to turnover ($p > 0.99$) or medical condition ($p = 0.35$), or due to the trap itself ($p = 0.08$), withdraw due to the loss of interest in the study was higher ($p = 0.005$) in low-income communities (50%) than in middle-income communities (5%).

3.2. Mosquito community composition

During the span of the study, we collected data from 3358 unique trap events, with each event representing the contents of a trap after being placed either indoors or outdoors for about one week. Among these events, 42.3% did not collect any mosquitoes (n = 1421) and 57.7% resulted in at least one mosquito (n = 1937). Among the trap events that did not capture mosquitoes, 87.1% were trap events that occurred indoor where the remaining 12.9% occurred outdoors. Among the trap event that captured mosquitoes, 77.2% occurred outdoors and 22.8% indoors. The average number of mosquitoes collected per trap event, regardless of placement or income, was 3.64. A total of 12,245 individual mosquitoes were collected among which 7875 mosquitoes were collected in the low-income communities and 4370 in the middle-income communities. 1048 mosquitoes were captured inside people's home and 11,197 outside. Finally, 10,832 of the mosquitoes were caught during the warm months versus 1413 during the cold months. The species composition in all sites consisted of *Ae. aegypti* (59.24%, n = 7255), *Culex* spp. (27.35%, n = 3350), *Aedes* spp. (2.0%, n = 245), *Ae. albopictus* (1.79%, n = 220), *Psorophora* spp. (0.16%, n = 20), *Anopheles* spp. (0.02%, n = 3), *Aedes vexans* (0.01%, n = 2), 4.32% (n = 530) were unidentified due to the poor condition of the specimen while 5.06% (n = 620) were of unknown species. As expected by using the AGO traps, female mosquitoes represented the majority of the mosquito specimens collected in the study (93.76%). Because of its dominance in the communities investigated and its medical importance regarding the transmission of ZIKV, DENV and CHIKV, the rest of the study focuses on *Ae. aegypti*. A total of 7255 female *Ae. aegypti* (96.3%) and 266 males (3.7%) were collected during our entire survey. Among all the 69 homes surveyed, 98.5% (n = 68) were positive at least once for the presence of female *Ae. aegypti* outdoors and 84.0% (n = 58) indoors. In low-income communities, 944% of the homes (n = 34) were positive for *Ae. aegypti* and 69.6% for middle-income communities (n = 23).

3.3. Aedes aegypti relative abundance

Aedes aegypti females were collected nearly every week during the span of the study. The overall average number of female *Ae. aegypti* collected per AGO per week was 1.59 with a range from 0 to 47.6 (Fig. 2). We used GLMM to investigate the importance of trap placement and income level on *Ae. aegypti* relative abundance. The best fit model explaining *Ae. aegypti* capture rates included the placement and income and the interaction between placement and income (Table S1), which revealed that the relative difference between indoor and outdoor capture rates was greater for middle-income communities compared to low-income communities (Table 3).

To investigate this interaction further, we compared the estimated margin means (emmeans) of the number of female *Ae. aegypti* mosquitoes according to income and placement. We compared for the indoor placement, the emmean of the number of mosquitoes in low-income communities (emmean = −0.99) and middle-income communities (−2.17) and for the outdoor placement, the emmean of the number of mosquitoes in low- (1.09) and middle-income communities (0.70). Our analysis, shows that the indoor mosquito population was higher in low-income communities when compared to middle-income communities (1.18 [0.17–2.19]) but that no statistical differences in the outdoor populations was observed according to income disparities (−0.39 [−0.6–1.38]).

Our model mirrored the raw data. Overall, collection of female *Ae. aegypti* was higher in outdoor settings with an average of 2.88 females per AGO trap per week whereas catches in indoor settings averaged at 0.30 females per AGO trap per week (Fig. 3-A). The outdoor mosquito density was 7.9 times higher than indoor mosquito density in low-income communities reaching 3.17 females per AGO trap per week. The outdoor/indoor ratio was even higher reaching 14.6 in middle-income communities with an average of 2.48 females per AGO trap per week (Fig. 3, Table 4). As mentioned above, income alone was a poor predictor for *Ae. aegypti* relative density (low = 1.78 females per AGO trap per week, middle = 1.32 females per AGO trap per week) and emphasize the importance of interaction between placement and income. Whereas outdoor mosquito populations did not significantly vary according to income (low-outdoor = 3.17; middle-outdoor = 2.48), significantly more (˜2.3 times) mosquitoes were found indoors in low-income communities (0.40 female per AGO per week) than in middle-income communities (0.17 female per AGO per week) (Fig. 3, Table 4).

The decline in temperature during the winter months correlated with a reduction in mosquito relative abundance (Fig. 2).

We used GLMM to investigate the importance of trap placement, seasonality, and their interaction on *Ae. aegypti* relative abundance. The best fit model explaining *Ae. aegypti* female variation capture rates included the interaction between placement and season (Table S2) and revealed that *Ae. aegypti* female patterns of seasonality is different indoors as compare to outdoors. The relative difference between indoor and outdoor capture rates was greater in warmer months than colder months (Table 5).

In order to understand which interactions were responsible for the changes in relative abundance between cold and warm seasons, we performed a comparison of the estimated marginal means. Our results show that the indoor mosquito population is not subject to seasonality (-0.85 [-0.10-1.80] (Fig. 3-B). For the outdoor population, our analysis revealed a significant difference in the outdoor populations between cold and warm season (1.11 [0.65–2.52]). In other words, seasonality was observed for the outdoor population (Fig. 3-B).

The model follows the patterns observed in the raw data with 1) more mosquitoes captured outdoor during the warmer months (3.52 female per SAGO per week) than during the cold months (0.86female per AGO per week) and 2) no difference in the number of mosquitoes captured indoor during the warmer months (1.00 female per AGO per week) compared to the winter months (0.85 female per SAGO per week) (Fig. 3, Table 4).

3.4. Arbovirus RNA testing of Ae. aegypti mosquitoes

In total, 6989 *Ae. aegypti* females were captured during the study. A subset of 1614 *Ae. aegypti* female pools representing 6910 mosquitoes were tested and found to be negative for both ZIKV and CHIKV using a multiplex RT-qPCR despite known controls being positive when performing this test.

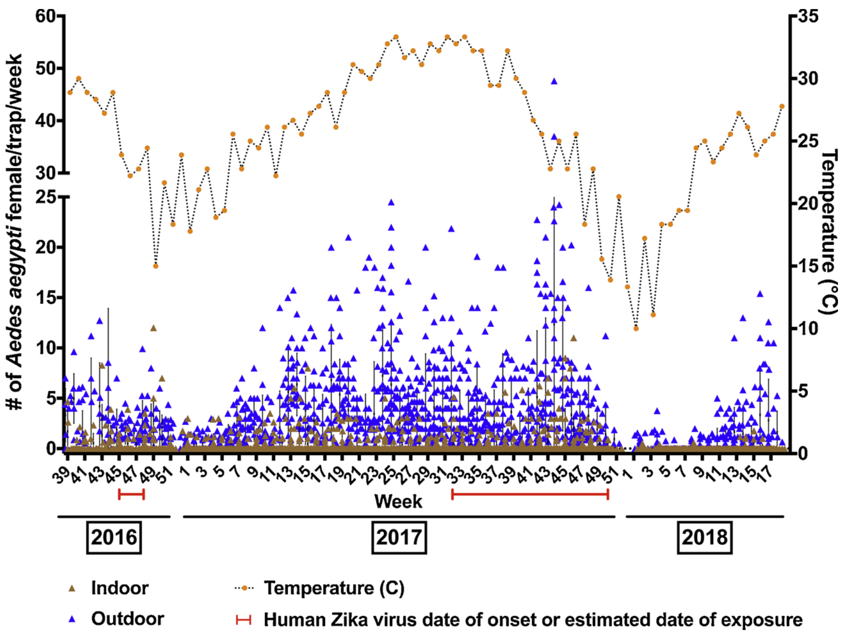

Fig. 2. Number of adult *Ae. aegypti* female mosquitoes collected per trap per week using Autocidal Gravid Ovitraps indoors and outdoors from September 2016 to August 2018 in eight communities in the Lower Rio Grande Valley. Mean weekly temperature (°C) is on secondary y-axis. Date ranges for local mosquito-borne human Zika cases are indicated under x-axis with blue lines.

Table 3
Parameters for best-fit model with the trap placement and community income interaction to explain variation in *Ae. aegypti* female mosquito relative abundance.

Variable	Coefficient	Standard error	Confidence Interval	
	–	–	Lower Bound	Upper Bound
Intercept	−3.36	0.36	−4.12	−2.62
Income (reference middle)	−1.18	0.40	−2.10	−0.30
Placement (reference outdoor)	2.09	0.08	1.94	2.24
Income*placement interaction	0.79	0.13	0.52	1.06
TrapID:community ID	−0.74	0.47	−1.00	−0.48
Month	0.00	1.00	−0.30	0.36
CommunityID	−0.67	0.51	−1.32	0.00

3.5. Zika cases in the LRGV

Sporadic low levels of endemic transmission have been reported for DENV and CHIKV in the LRGV. Concurrent with this study, the county and state health departments identified ten human ZIKV symptomatic infections (within various cities of Hidalgo County including Alamo, McAllen, Mercedes, and Pharr) that were locally acquired by mosquito-bites based on investigations concurrent with the current study in the LRGV. Six of these occurred in Brownsville, TX (Cameron County) in November 2016 with a date of onset case one and two during week 45, week 46, and four remaining cases in week 48 (Fig. 2). All cases were symptomatic. Four locally acquired infections occurred in Hidalgo County, TX. This included 4 cases of symptomatic disease. Of the four symptomatic cases, date of symptom onset ranged from August 2017 to December 2017. Onsets of cases were week 32, 38, 40, and 50. The estimated ZIKV date of onset of disease or exposure to mosquitoes for these locally-acquired cases occurred during periods of elevated *Ae. aegypti* relative abundance (Fig. 2).

4. Discussion

In order to prevent the spread and/or the re-emergence of arboviral diseases in the U.S., it is important to understand the local risk factors.

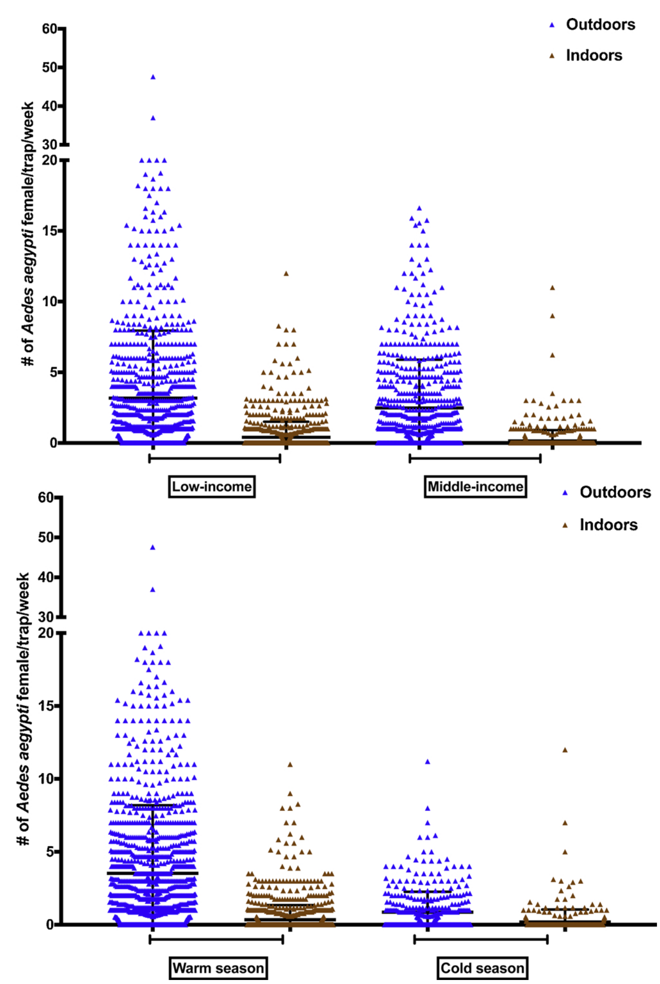

Fig. 3. Number of adult *Ae. aegypti* female mosquitoes collected per trap per week using Autocidal Gravid Ovitraps indoors and outdoors according to A) socio-economic status and B) season.

Table 4
Summary of female catch per trap per week for *Aedes aegypti* in indoor and outdoor settings according to income and season.

	Aedes aegypti f/trap/week Mean ± SD (N)	
	INDOOR	OUTDOOR
Low-income	0.40 ± 1.09 (528)	3.17 ± 4.77 (4008)
Middle-income	0.17 ± 0.73 (149)	2.48 ± 3.41 (2304)
Cold season	0.19 ± 0.85 (114)	0.86 ± 1.39 (583)
Warm season	0.34 ± 1.00 (563)	3.52 ± 4.66 (5729)
Total	0.30 ± 0.97 (677)	2.88 ± 4.27 (6312)

Table 5
Parameters for best-fit model with the trap placement and seasonality to explain variation in *Ae. aegypti* female mosquito relative abundance.

Variable	Coefficient	Standard error	Confidence Interval	
	–	–	Lower Bound	Upper Bound
Intercept	−4.34	0.39	−5.15	−3.56
Season (reference warm)	0.84	0.39	0.05	1.65
Placement (reference outdoor)	1.72	0.14	1.44	2.01
Season*placement interaction	0.75	0.16	0.43	1.06
TrapID:community ID	−0.75	0.47	−1.00	−0.48
Month	−0.29	0.55	−0.61	0.08
CommunityID	−0.56	0.32	−1.15	−0.09

The present study documents the relative abundance of indoor and outdoor *Ae. aegypti* captured during a surveillance program and the heterogeneity due to seasonality and income level. Overall, *Ae. aegypti* was the most abundant mosquito species found in the eight communities surveyed follow by *Culex* spp. with rare observations of *Ae. albopictus*. Presence of a mosquito vector species is one of the requirements for arbovirus transmission to occur and mosquito distribution maps are necessary to assess the global distribution and the predictive range of these vector species. As of March 2016, *Ae. aegypti* has been reported in 26 states with the highest abundance in Florida, Texas, Arizona and California (Hahn et al., 2016). The scarcity of *Ae. albopictus* in South Texas highlighted by the current study is confirmed by the recent distribution maps (Hahn et al., 2017; Johnson et al., 2017), showing that this region of Texas is the western edge of the distribution of *Ae. albopictus*, except for populations in California and scattered populations throughout Mexico. The long-term relative abundance of these two mosquito species in South Texas is poorly documented thus far with two prior studies reporting variation in abundance for both *Ae. aegypti* and *Ae. albopictus* in the LRGV (Champion and Vitek, 2014; Vitek et al., 2014).

In this study, we used the CDC AGO trap as a surveillance tool to understand baseline mosquito abundance in eight communities in the LRGV. We detected *Ae. aegypti* inside 84.0% of the homes investigated while 98.5% of the homes had *Ae. aegypti* in the outdoor AGOs. The CDC AGO trap was first developed and used in Puerto Rico in outdoor settings. Researchers there reported an average abundance of *Ae. aegypti* females per trap per week ranging from 2.65 to 3.78 females per AGO per week in pre-intervention communities (Barrera et al., 2014a). Our outdoor relative abundance results in South Texas communities are in concordance with the Puerto Rican studies. When we look at the results by income level, we see that the average catch in outdoor traps for female *Ae. aegypti* in low-income communities is 3.13 per trap per week and in middle-income communities is at 2.46 per trap per week. These values are close to the threshold value of 3 females per AGO per week above which the risk for CHIKV transmission in Puerto Rico is high (Barrera et al., 2017; Lorenzi et al., 2016). Other studies in Puerto Rico have revealed higher *Ae. aegypti* abundance with variation according to community (Barrera et al., 2014b). The difference in the average number of mosquitoes collected could be due to the different amount of hay use to bait the AGO traps. Indeed, in our study, only a tenth of the recommended hay concentration could be use due to the requirement to place the trap inside people's home. Additionally, this modification could explain the moderate efficiency (57.7%) of the AGO trap at catching mosquitoes. Among the positive AGO capture events we found that 77.2% occurred outdoor vs 22.6% indoor. Here, we provide the first evidence that AGO traps are able to target *Culex* spp. The lower relative abundance of *Culex* mosquitoes collected in this study compare to *Ae. aegypti* might reflect the lower efficiency of AGO traps toward capturing these mosquito species that exhibit preference towards organically rich oviposition sites (Cilek et al., 2017).

Estimating adult mosquito relative abundance using a standardized index has rarely been done indoors and outdoors and this study takes a step in utilizing a recently developed AGO trap to understand risk of human exposure to mosquito-borne viruses in these two contrasting environments. Our present study demonstrates the occurrence of adult *Ae. aegypti* both outdoors and indoors nearly every week of the calendar year. We found that, on average, the indoor relative abundance of *Ae. aegypti* was lower when compared to the outdoor relative abundance. Additionally, the difference in mosquito relative abundance was lower between outdoor and indoor in low-income communities (˜8-fold) compared to middle-income communities (˜14.5-fold). These observations highlight income as a risk factor for indoor relative abundance of mosquitoes. Our current study also shows that outdoor mosquito relative abundance is not necessarily different according to income level but it is the ability of mosquitoes to enter and/or live indoors that is higher in lower income communities. The presence of *Ae. aegypti* indoors is of concern as this could reflect an increased risk for human-vector contact and thus increased disease occurrence in residents of low-income communities. Indeed, income has been previously identified as a factor influencing arboviral transmission. For example, a previous study conducted along the Texas-Mexico border identified income as a risk factor for both past and recent DENV infection, together with the presence of larval habitats, the absence of air conditioning, and absence of street drainage (Brunkard et al., 2007; Reiter et al., 2003). In Brazil, a negative correlation between DENV seroprevalence and income was demonstrated (Braga et al., 2010; Siqueira et al., 2004). Numerous studies have demonstrated the role of socioeconomic factors such as level of education, household infrastructure and crowding conditions as well as water access and sanitation to contribute to *Ae. aegypti* mosquito infestation and dengue disease seroprevalence (Sallam et al., 2017; Siqueira-Junior et al., 2008; Spiegel et al., 2007; Zellweger et al., 2017). The current study identifies the need to further understand socio-economic factors responsible for variation in mosquito relative abundance between the low- and middle-income communities and to consider the indoor mosquito population during vector control activities in the LRGV. Although targeted indoor mosquito control for *Ae. aegypti* is becoming more common in much of the world, including Australia (Vazquez-Prokopec et al., 2017) and Peru (Gunning et al., 2018), these activities are not routinely done in the continental U.S.

Climatic conditions can also impact multiple entomologic factors that have potential effects on arboviral disease transmission. Our study shows seasonal variation in *Ae. aegypti* relative abundance with more mosquitoes captured during the warmer months. For *Ae. aegypti,* a 4-fold increase in outdoor populations was demonstrated. Similarly, *Ae. aegypti* outdoor populations in Puerto Rico and Brazil were also positively associated with temperature (Barrera et al., 2011; Degener et al., 2014). Increase in temperature is linked to a faster development of immature stages of mosquitoes and increased growth of microorganisms on which mosquito feed resulting in a higher density of mosquitoes (Grech et al., 2015; Rueda et al., 1990; Tun-Lin et al., 2000). Additionally, higher temperatures result in a reduced gonotrophic cycle with females biting and laying eggs more frequently (Arruda Pedrosa de Almeida Costa et al., 2010; Marinho et al., 2016).

As opposed to outdoor mosquito populations, *Ae. aegypti* indoor populations were not subject to seasonality with a similar number of mosquitoes observed indoor during the warm and the cold months. This observation emphasizes that mosquitoes in the indoor environment are subjected less to ambient conditions and this could help them persist during the colder months. Future longitudinal studies will be designed to assess the impact of weather (e.g., temperature range, precipitation, relative humidity) on *Ae. aegypti* mosquito relative abundance and arboviral dynamics in the LRGV (Ernst et al., 2017; Joy et al., 2012).

The low level of participation represents the major limitation of our study and could have introduced a bias such that the data collected from participating homes was not representative of the rest of the community. Of the 349 houses visited in the eight communities within the LRGV in South Texas, most of the households were unavailable at the time of the three visits attempted (65%). This "unavailability" was mainly linked to the true absence of the home owner at the time of the visit but also to other factors such, abandoned households, fenced properties or presence of aggressive dogs restricting interaction with home owners, as well as denial by home owners. Of the 123 responding households, about half of them agreed to participate in the surveillance study (56%) and the other half denied access to their properties. Among the participants, 52% withdrew from the study before the end of the study (Table 2). The main reason for withdrawing from the study was associated to loss of interest (significantly higher for low-income communities) and concern about traps (e.g. smell and danger for children). In a similar study done in Merida, Mexico, but using a backpack aspirator as a collection method, receptivity of the public to the study was higher with 70.8% of enrollment, 4% denied access, and 25.2% of unoccupied household (Garcia-Rejon et al., 2008). Beside the reasons stated above, the political climate and the tighter immigration laws could be underlying factors explaining at least in part the low response and participation rate. The high percentage of houses unavailable at the time of the visits is a potential problem if we want to pursue future vector control intervention in these communities and highlight the importance of establishing early contact and trust with the local population (Andersson et al., 2015). Alternatively, collaboration with local health departments or local leaders could facilitate community engagement (Gubler and Clark, 1994; Lloyd et al., 1992).

5. Conclusions

Our study reports how income level and temperature influence mosquito population dynamics in South Texas, which can be used to implement more efficient vector control measures for the benefit of public health in the LRGV. Other socio-economic factors relevant at the household and area level should be further investigated, as well as climatic and entomological factors such as feeding and biting behavior and co-infection status of mosquito populations. Additionally, a vector control intervention will be implemented over the course of two years using the AGO to reduce mosquitoes in these same communities utilizing a similar study design previously used in Puerto Rico (Lorenzi et al., 2016).

Author summary

The yellow fever mosquito (*Aedes aegypti*), the Asian tiger mosquito (Aedes albopictus) and *Culex* spp. mosquitoes are vectors of major mosquito-borne viral diseases reported in the United States, including Texas. The relative abundance of outdoor mosquito populations with those that enter human homes is an important factor of mosquito-human contact and thus risk of virus transmission. In this study, we used CDC Autocidal Gravid Ovitraps (AGO) as sentinels to monitor the mosquito relative abundance indoors and outdoors at weekly intervals between September 2016 to April 2018 in low- and middle-income communities in the Lower Rio Grande Valley (LRGV) of South Texas. We report longitudinal patterns of *Ae. aegypti* highlighting the presence of adult mosquitoes nearly every week of the year. Overall, the relative abundance of mosquitoes' outdoors was higher than indoors and low-income communities had more mosquitoes indoors compared to middle-income communities, suggesting that social factors such as home integrity might influence the ability of mosquitoes to enter homes. Ten human Zika cases were acquired locally in the LRGV by mosquito bites concurrent with this study. Together, this study serves as basis to implement mosquito control in a high-risk area for arboviral disease emergence in the United States.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.actatropica.2019.02.006.

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