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## Defining *Aedes aegypti* density thresholds for preventing human arboviral infections

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### Abstract

Effective control of local dengue virus transmission requires reducing the population of mosquitoes below a critical density threshold. *Aedes aegypti* density thresholds can serve as targets for intervention effectiveness. Most thresholds have been identified by correlating vector densities with pathogen transmission events. However, despite previous descriptive research there is a need for rigorously controlled field studies to clarify the interactions between *Ae. aegypti* density and human infections. We report on a multi-arm cluster randomized field trial aimed at determining the number of female *Ae. aegypti* mosquitoes below which dengue outbreaks are limited. We implemented four mosquito control treatments in a dose-response trial, using zero to three Autocidal Gravid Ovitrap (AGO traps) per house across study neighborhoods in Caguas, Puerto Rico from January 2021 to June 2024. We anticipated that significant reductions in *Ae.*

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*aegypti* populations would indicate a threshold density if low or no prevalence of dengue viruses was observed in mosquitoes or antibodies in residents through IgM and IgG serological testing. Results showed that female *Ae. aegypti* densities decreased by 44%, 60%, and 80% in clusters with one, two, and three traps/house, respectively. The expected number of dengue-infected mosquitoes per thousand were 2.01, 1.70, 0.60, and 0.36 in clusters with zero, one, two, and three traps/house, respectively. Increases in dengue IgG antibody prevalence from 2022 to 2023 were noted only among residents aged 1–10 years, with rates of 25%, 18%, 6%, and 5.9% corresponding to the number of traps used. To reduce dengue incidence, local populations of *Ae. aegypti* should be kept below 4 females per AGO trap per week.

## Keywords

*Aedes aegypti*; dengue; mosquito surveillance; mosquito control; threshold density; cluster randomized trial

## 1. Introduction

*Aedes aegypti*, the main vector of arboviruses such as yellow fever, dengue, chikungunya, and Zika was eliminated during the 1950s - 1960s from most countries in Latin America to forestall epidemics of urban yellow fever (PAHO 1980). Despite these successes, *Ae. aegypti* has since reestablished itself across the region, and no country has achieved its permanent elimination. Its resurgence has facilitated the spread of dengue, chikungunya and Zika viruses, which are now the foremost arboviral diseases afflicting the Americas. The rising incidence of dengue (PAHO 2024) highlights the limitations of shifting from eradication strategies to mosquito control.

The *Stegomyia* or *Aedes* threshold indices (House Index: 1–5% houses with at least one positive container, Container Index: <10% positive containers, and Breteau index: <5 positive containers per 100 houses) (Connor and Monroe 1923, WHO 2009) that were originally developed from empirical observations during yellow fever epidemics have been proposed as benchmarks for preventing dengue epidemics. These indices, which rely on detecting larvae or pupae in household containers are more suited to elimination efforts focused on vector presence rather than abundance. Although some studies have demonstrated correlations between these indices and reduced dengue risk (Pontes et al. 2000, Sanchez et al. 2006), others have not (Goh 1997, Bowman et al. 2014, Bajwala et al. 2020). The increased discovery of cryptic aquatic habitats of *Ae. aegypti*, often undetectable during inspections, likely contributes to underestimations of its prevalence and abundance (Barrera et al. 2008, Koh et al. 2008).

The advent and use of efficient traps to capture adult *Ae. aegypti* provide a more direct measure of arbovirus transmission risk, as high mortality rates of immature stages often result in weak correlations between larval counts and emerging adults capable of transmitting viruses (TunLin et al. 1996, Focks and Chadee 1997, Focks 2003). Efforts to establish more reliable mosquito density thresholds have focused on adult *Ae. aegypti*. Several studies have reported density thresholds associated with low dengue virus transmission, though these thresholds vary depending on trap type, environmental

conditions, and other factors (Ritchie et al. 2004, Eiras and Resende 2009, Barrera et al. 2017, Sharp et al. 2019). No universal threshold exists, as values depend on variables such as type of mosquito trap, virus introduction frequency, herd immunity, temperature, and protection against mosquito bites (Focks et al. 2000, Aryaprema et al. 2023). In Puerto Rico, where dengue viruses have been endemic for decades, high levels of population immunity and frequent virus introductions pose unique challenges for defining actionable mosquito density thresholds.

Establishing actionable mosquito density thresholds could enable targeted vector control strategies, optimize resource allocation, and enhance the efficacy of arbovirus prevention programs, particularly in regions with limited public health resources. Determining actionable *Ae. aegypti* density thresholds to prevent or control dengue transmission has been based largely on empirical observations and correlations between qualitative or quantitative mosquito indices and disease incidence. However, Scott, Morrison, and Takken (Scott et al. 2003) noted, “no well-controlled field studies have been carried out to define the dynamic relationships between *Ae. aegypti* density and human virus infections”. A potential experimental approach to identify and validate such thresholds involves maintaining mosquito populations at different stable levels over extended periods to observe variations in arbovirus transmission. This method, though untested, would require sustained and effective mosquito control interventions applied in a dose-response fashion under natural urban conditions.

Here we report the findings of a multi-arm cluster randomized field trial aimed at establishing the threshold density of female *Ae. aegypti* mosquitoes below which dengue outbreaks are limited. Four mosquito control treatments were implemented in a dose-response framework, with study neighborhoods assigned varying levels of control intensity by deploying zero, one, two, or three Autocidal Gravid Ovitrap (AGO traps; as described below) per house across most households (Barrera et al. 2014b, Barrera et al. 2014a) over a three-year period. We hypothesized that neighborhoods with higher AGO trap densities would sustain significantly lower mosquito populations, leading to reduced dengue virus prevalence in both vectors and human residents.

## Methods

### Ethics Approval and Consent to Participate

This investigation received Institutional Review Board approval (*Aedes aegypti* density threshold preventing human arboviral infections; #1910024340R004; Ponce Research Institute, Ponce Medical School Foundation, Inc.). Participants in the serology study were 1 – 51 years old. Participation in this study was voluntary and participants were informed that they can withdraw at any time. Written informed consent was obtained from all study participants aged ≥ 21 years. Caregivers provided written informed consent for participants aged < 21 years. Oral assent was obtained from participants aged between 7 and 13 years and written assent from participants aged between 14–20 years.

## Experimental design

The study was conducted in Caguas City, Caguas Municipality (18.23412 N, -66.0485 W), Puerto Rico, from January 2021 to June 2024 (Figure 1). Averages ( $\pm$  Std. Error) of weekly rainfall, daily temperature, and daily relative humidity during the study were  $26.99 \pm 1.52$  mm ( $n=751$ ),  $26.53 \pm 0.025$  °C ( $n=5262$ ), and  $80.28 \pm 0.08$  % ( $n=5262$ ), respectively. It was designed as a four-arm, cluster-randomized trial with three replicates per arm, involving approximately 300 houses per cluster (3610 houses across 12 clusters; Supplementary Table 1). The number of houses per cluster was chosen given available resources. The four treatments included the deployment of zero, one, two, or three AGO traps per house across most houses within each cluster (Figure 1). AGO traps were used to control *Ae. aegypti* based on previous effectiveness studies conducted in Puerto Rico (Barrera et al. 2014a, b; 2017; 2019a, b). Throughout the manuscript we will refer to AGO traps as IAGO or SAGO traps. Although intervention AGO traps (IAGO) and surveillance AGO traps (SAGO) are identical in design, they served different purposes: mosquito counts were conducted exclusively in SAGO traps, while the more numerous IAGO traps were used solely for intervention.

The primary outcome was the number of female *Ae. aegypti* per surveillance trap per week. The hypothesis suggested that an increase in the number of traps per house would lead to a significant reduction in the average number of female *Ae. aegypti* mosquitoes and consequently result in lower dengue virus prevalence among mosquitoes and dengue antibodies in residents. Secondary outcomes included the prevalence of infected female *Ae. aegypti* mosquitoes and changes in dengue seroprevalence among a sample of residents from a first serosurvey in 2022 to a second serosurvey in 2023. To assess whether *Ae. aegypti* populations rebounded after trap removal, mosquito abundance was monitored in eight randomly selected clusters (out of the original 12). Trap removal occurred over two months (January–February 2024), with post-intervention data collected from March to June 2024 (Figure 2).

Before initiating the study, we engaged with participating communities and the Caguas municipality to explain the trial's purpose and seek their collaboration. Study clusters were selected based on similar historical averages of female *Ae. aegypti* per trap per week, as well as comparable land use characteristics, and human population densities (Supplemental Table 1). Mosquito abundance data were sourced from a previous surveillance program conducted by the Puerto Rico Vector Control Unit in Caguas City between June and November 2018, which used AGO traps. Human population and land use information were derived from the 2012 Territorial Plan of Caguas City. The 12 clusters were randomly assigned to one of the four treatments using Python's "random" library (<https://python.readthedocs.io/en/stable/library/random.html>).

Traps were deployed in the treatment clusters between January and April 2021 (Figure 2). Surveillance traps were installed in late April 2021, with the first weekly report on female *Ae. aegypti* abundance per trap generated in May 2021. In each cluster, 20 SAGO traps were evenly distributed, fixed at consistent locations, and monitored weekly. Mosquitoes collected in SAGO traps were counted, and pooled samples were prepared for virus identification. The average distance between SAGO traps within clusters was about 60.7 meters (1.3 traps/Ha),

calculated using the Average Nearest Neighbor tool in ArcGIS Pro (ESRI, Redlands, CA). The number of IAGO traps varied by treatment, with 226 (12.8 traps/Ha), 446 (29.8 traps/Ha), and 655 (46.6 traps/Ha) traps per cluster in treatments with one, two, and three traps per house, respectively. The percentage of houses with traps ranged from 72–78%, 74–75%, and 68–74% for clusters with one, two, and three traps per house respectively.

AGO traps use sticky glue to capture gravid female mosquitoes attracted to the trap for oviposition. Each trap consists of a black plastic bucket containing ten liters of water and a 30-gram packet of hay grass, which decomposes to produce odors that lure female mosquitoes. The capture chamber includes a fine mesh screen that prevents mosquitoes from reaching the water. Every week, trapped mosquitoes were removed from the sticky glue, placed on paper towels, and identified by sex and species in the field by trained technicians. Both SAGO and IAGO traps were installed outdoors and serviced every two months, during which the traps were cleaned, and water, hay packets, and sticky boards were replaced (Barrera et al. 2014b).

### Detection of dengue viruses in *Ae. aegypti*

Several previous studies have shown that viral RNA of DENV, CHIKV, and ZIKV can be detected in dead mosquitoes that have been exposed at room temperature or in the field for over one week (Barrera et al. 2019a). Female *Ae. aegypti* mosquitoes collected from SAGO traps were pooled weekly into groups of 1–20 mosquitoes per vial and preserved at –20°C until processed for dengue, chikungunya, and Zika virus detection. To prevent contamination, dissecting needles were sterilized between transfers from individual traps to vials. Viral RNA was identified using the CDC’s triplex RT-PCR assay (Santiago et al. 2018). To optimize testing efficiency, we created super pools by combining 50µl aliquots from each of ten individual pools into a single 500µl sample. If a super pool tested positive for dengue virus, each constituent pool was individually tested by RT-PCR to determine the presence of the virus and to identify the dengue serotype (DENV-1, DENV-2, DENV-3, or DENV-4) (Santiago et al. 2013). If a super pool tested negative, no further testing was performed.

### Serological studies

To assess whether residents in the study areas were differentially infected with dengue viruses according to treatment during part of the investigation, we carried out two serosurveys. The first took place from June to September 2022 and the second from October to December 2023. Phlebotomists and interviewers collected 50 finger-prick blood samples from each of the 12 clusters, resulting in a total of 600 tests per serosurvey. When the 50 blood samples were obtained in each study cluster, we stop recruiting participants. These samples were tested on-site for immunoglobulin M (IgM) antibodies using the CTK Biotech OnSite Dengue IgG/IgM Combo Rapid Test and for immunoglobulin G (IgG) antibodies using the CTK Biotech OnSite Dengue IgG Rapid Test (<https://ctkbiotech.com/onsite-dengue-igg-igm-combo-rapid-test-ce/>). During household visits to the clusters, eligible participants were identified after the purpose of the study was explained and their consent to participate was obtained. Participants were eligible if they met the following criteria: residence in one of the study areas, age 1–50 years for the first serosurvey or 1–51 years

for the second, provision of informed consent, completion of a short questionnaire, and agreement to provide a blood sample.

### Dengue cases

Georeferenced data of confirmed dengue cases and circulating virus serotypes for Caguas municipality were obtained from the Puerto Rico Health Department.

### Statistical methods

Differences in the average number of female *Ae. aegypti* per trap per week among treatment arms were analyzed using a Generalized Linear Mixed Model, with a negative binomial distribution. The model included an autoregressive order-one covariance structure to account for repeated measures and incorporated fixed effects for accumulated rainfall (two and three weeks prior to sampling) and the average daily air temperature and relative humidity over the previous three weeks. Random effects were specified for cluster and trap ID. The analyses were done using the `glmmTMB` package in R (<https://cran.r-project.org/web/packages/glmmTMB/index.html>).

To test if there were significant differences in the average number of *Ae. aegypti* females per trap per week after trap removal between control and treatment clusters, we extended the GLMM described above by including an interaction term between treatment levels (0, 1, 2, or 3 traps per house) and trapping period (pre-trap removal, trap removal, and post-trap removal). The model evaluated whether changes in *Ae. aegypti* density during and after trap removal differed across treatment groups. Data for the pre-trap removal (September–December 2023) and post-trap removal (March–June 2024) periods were analyzed over four months, while data from the transitional trap removal period (January–February 2024) spanned two months.

Minimum dengue infection rates of pooled *Ae. aegypti* females were calculated using the “PooledInfRate”1.6 package in R (<https://github.com/CDCgov/PooledInfRate>). The minimum infection rate assumes that if a pool is positive for the virus, then at least one mosquito in the pool was positive. It is calculated by dividing the number of positive pools by the total number of mosquitoes tested. The Vector Index (VI), or number of expected infected mosquitoes per thousand, was calculated as the product of the infection rate times the average number of *Ae. aegypti* per trap per week (Jones et al. 2011). To calculate confidence intervals, we used non-parametric bootstrapping with 10,000 iterations and resampling data with replacement. The 95% CIs were obtained using the percentile method, capturing the middle 95% of the resampled differences. The package used in R for estimation was boot 1.3–31 (<https://cran.rstudio.com/web/packages/boot/index.html>).

We also used GLMMs to explore potential statistical differences in IgM and IgG antibody prevalence across treatments and demographics. These models assessed whether seroprevalence significantly varied by treatment, survey year, age group, sex, the interaction between age group and year, and average weekly hours spent in the household. The GLMMs were specified with a binomial distribution and a logit link function. Cluster-level variability was addressed by including cluster as a random effect to control for intra-cluster correlation.

Models were fit using the `glmer` function in the R `lme4` package (<https://github.com/lme4/lme4/blob/master/man/glmer.Rd>).

To estimate confidence intervals for the difference in IgG seroprevalence between 2022 and 2023 within each treatment group, we used non-parametric bootstrapping with 10,000 iterations. For each iteration, we resampled the observed data with replacement and computed the difference in seroprevalence proportions. The 95% CIs were obtained using the percentile method, capturing the middle 95% of the resampled differences. This approach avoids normality assumptions and is more robust for small sample sizes. We used the `boot()` function from the `boot` package for bootstrapping. Statistical analyses were conducted using R software version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

## Results

### Treatment effects

The average numbers of female *Ae. aegypti* captured per trap per week decreased as the number of IAGO traps per house increased, from  $9.68 \pm 0.09$  (95% CI; N= 8279 trap-weeks) in clusters without IAGO traps to  $5.48 \pm 0.06$  (N= 8274),  $3.92 \pm 0.04$  (N= 8295), and  $2.15 \pm 0.03$  (N= 8272) in clusters with one, two, and three IAGO traps per house, respectively (Figure 3). GLMM analysis indicated significant effects of treatment (number of IAGO traps per house), rainfall, temperature, and relative humidity ( $p$ -values < 0.001; Table 1). Compared with control clusters, reductions in the average number of female *Ae. aegypti* were 43.6% (95%CI: 23.5 – 58.5), 60.3% (46.2 – 70.8), and 79.6% (72.3 – 85.0) in clusters with one, two, and three traps per house, respectively. Weekly fluctuations in *Ae. aegypti* counts were smaller in clusters with higher trap-house densities, with short-term increases primarily associated with increases in rainfall (Figure 3).

After the removal of IAGO traps in January and February of 2024, *Ae. aegypti* populations increased in the experimental clusters compared to the control clusters (Figure 4). Average numbers of *Ae. aegypti* per trap per week changed from 13.4 to 11.2 in control clusters, 6.8 to 10.1 in clusters with one trap/house, 4.3 to 9.6 in clusters with two traps/house, and 2.5 to 8.1 in clusters with three traps/house (Table 2). GLMM analysis revealed significant interactions ( $p$  < 0.001) between treatment intensity (0, 1, 2, or 3 traps per house) and trapping period (pre-removal, during removal, and post-removal (Supplemental Table 2). The interaction effects were driven by a slight decrease in female *Ae. aegypti* numbers in control clusters as compared to marked increases in treated clusters after trap removal (Figure 4).

### Detection of dengue viruses in *Ae. aegypti*

During the study, 39 dengue virus detections were made in female *Ae. aegypti* captured in SAGO traps, comprising four instances of DENV-1 and 35 instances of DENV-2. Dengue-positive pools were identified across clusters, with 17 positive pools in control clusters and 14, 5, and 3 positive pools in clusters with one, two, and three traps per house, respectively (Table 3). Overlapping 95% confidence intervals for minimum infection rates

between treatments indicated no statistically significant differences ( $p > 0.05$ ; Table 3). The corresponding Vector Index values (95% C.I.) were 2.01 (1.01–2.92), 1.70 (0.71–2.54), 0.60 (0.11–1.09), and 0.36 (0 – 0.73) for control clusters and clusters with one, two, and three traps per house, respectively. These findings suggest lower numbers of infected mosquitoes (Vector Index) in clusters with three traps compared to controls and those with only one trap per house.

### Dengue cases and prevalence of IgG antibodies in residents

There were 126 confirmed dengue cases in Caguas municipality during the study period, of which 31 were DENV-1, 42 DENV-2, 18 DENV-3, and 36 unidentified. The number of reported dengue cases in the 12 study clusters was small (3 – 5 cases/treatment clusters) and similar across treatments.

Participation of residents was uniform across both serosurvey years, with 50 participants per cluster and 150 participants per treatment group. The sex distribution was consistent between the two serosurveys, with 53.5% female and 46.5% male participants in the first serosurvey, and 53.7% female and 46.3% male participants in the second serosurvey. The age distribution also showed similar patterns across both serosurveys. IgM test positivity was low across clusters during the first (2.7 – 6.0%) and second (0 – 2%) year. IgG test positivity was similar and high during the first (67 – 74%) and second (68 – 73%) year.

Distinct differences in IgG seroprevalence were observed across age groups, indicating a trend of increasing seroprevalence with age (Figure 5). The results of the GLMM analysis revealed no significant differences ( $p > 0.05$ ; Supplemental Table 3) in overall IgG seroprevalence between intervention groups (one, two, or three traps/house compared to controls). Additionally, serosurvey year did not have a significant impact, as the estimated overall seroprevalence remained consistent in both years. Sex and average weekly hours spent in the household were also not significant predictors (Supplemental Table 3). The primary factor associated with past dengue infection in our study population was age group.

The statistical interaction of the age group and serosurvey year was significant ( $p < 0.05$ ), reflecting that increases in IgG prevalence was observed only among participants 1–10 years old (Figure 5; Supplemental Table 3). Bootstrapped 95% confidence intervals for the difference in IgG seroprevalence between 2022 and 2023 were calculated for each treatment group. The largest increase (95% C.I.) was observed in the control group (25%; 0.00 – 0.50), followed by clusters with one trap per house (17.8%; –0.13 – 0.49), two traps (5.7%; –0.19 – 0.30), and three traps (4.9%; –0.14 – 0.33). The overlapping confidence intervals across treatment groups suggest that changes in seroprevalence were not significantly different between treatment groups (Figure 5).

### Discussion

This investigation aimed at controlling the populations of *Ae. aegypti* in clusters of residential neighborhoods with increasing levels of mass-trapping, from clusters with no mass-trapping (control clusters) to treatment clusters with one, two, and three AGO traps/house in most of the houses. The goal was to find threshold mosquito densities that

minimized infections with dengue virus in residents and mosquitoes. The results showed that the relative abundance of *Ae. aegypti* significantly decreased with increasing trap density, with maximal reductions of 80% in clusters with three traps/house. Also, weekly fluctuations in the relative density of *Ae. aegypti* females were smaller in clusters with more traps/house, producing smaller and steadier populations throughout the study. The impact of mass trapping on the relative density of *Ae. aegypti* was further demonstrated by the significant increases observed after the intervention traps were removed from treatment clusters. Similar magnitude of reductions in the relative abundance of *Ae. aegypti* females were observed in previous studies in Puerto Rico (Barrera et al. 2014b, Barrera et al. 2017, Barrera et al. 2019a, Barrera et al. 2019c), Mexico (Aguilar-Durán et al. 2024), and South Texas (Juarez et al. 2021) where mass-trapping was conducted with three AGO traps/house. Observations from these studies emphasize two key factors critical to the success of mass trapping: the number of traps deployed per building and the proportion of buildings in the area equipped with traps.

Previous studies conducted during the chikungunya (Sharp et al. 2019) and Zika (CDC, unpublished) virus epidemics demonstrated significant reductions of approximately 50% in IgG antibodies among residents of neighborhoods utilizing mass AGO trapping. This reduction was observed in areas where the relative abundance of *Ae. aegypti* was below three females per trap per week. Because people in Puerto Rico had not experienced previous epidemics of chikungunya or Zika viruses, the lack of population immunity to these viruses implies that fewer mosquitoes (lower threshold) would be needed for local transmission than for viruses such as dengue that have circulated in Puerto Rico for decades (Focks et al. 2000, Barrera 2010). If a percentage of the human population has antibodies against the circulating arbovirus, more mosquitoes will be needed to cause an outbreak because a portion of the infective bites would go into people who are already immune. Consistent with this, our study showed high dengue seroprevalence that increased with age in the study areas. Consequently, we expected that higher mosquito densities would be required to sustain dengue virus transmission. Low circulation of dengue viruses during the study precluded acquiring stronger evidence to signal a minimum density of *Ae. aegypti* females that would be protective against local dengue virus transmission. Nevertheless, we observed smaller number of expected infected mosquitoes or vector indices in neighborhoods with two and three AGO traps per house. We also observed increases in dengue antibodies in younger residents (1–10 years old) in neighborhoods without mass trapping (25%). By contrast, we did not observe any changes in antibody prevalence in older people. Older age groups exhibited a high prevalence of dengue antibodies at baseline, while the younger population showed lower seroprevalence due to reduced exposure to dengue transmission over the past decade in Puerto Rico (Adams et al. 2023).

The average densities of *Ae. aegypti* females in neighborhoods with lower prevalence of dengue viruses in mosquitoes and lower prevalence of dengue antibodies in younger residents were 2.15 – 3.92 mosquitoes per trap per week in areas under treatment with three and two traps per house, respectively. These results suggest that keeping the *Ae. aegypti* population below four females per AGO trap per week can be considered a protective density threshold against dengue virus infections under current epidemiological conditions, which corresponds to 80–60% reductions in the mosquito population.

To better understand the degree of reduction in *Ae. aegypti* populations that would normally be necessary to achieve the density threshold, it is useful to review the densities of this mosquito observed in previous studies in areas without mosquito control. The numbers of *Ae. aegypti* females in study clusters before the AGO trap intervention were 8–16 per AGO trap per week. The numbers of *Ae. aegypti* females in AGO traps where Zika and chikungunya viruses were detected in mosquito pools during 2016–2017 in Caguas city was 9.8 and 10.3, respectively (Barrera et al. 2019c). Studies conducted in two untreated neighborhoods of southern Puerto Rico between 2014 and 2017 showed that the average densities of *Ae. aegypti* females per AGO trap per week were 10 – 14 in one community and 7 –15 in the other community (Barrera et al. 2014a, Barrera et al. 2019b). These average values from field studies using the same surveillance tool (AGO trap) show that the density of *Ae. aegypti* in several sites and epidemiological situations in Puerto Rico are above the threshold densities that would be required to lower the risk of local transmission of dengue viruses (2 – 4 mosquitoes per trap per week).

The low circulation of dengue viruses during this investigation limited our capacity to better assess changes in dengue antibodies in residents and the prevalence of viruses in mosquitoes. Mosquito pool infection rates detected in this study (0.154 – 0.310) were much lower than those previously observed during chikungunya (1.754 – 2.483) and Zika (1.205 – 2.064) outbreaks in Puerto Rico (Barrera et al. 2019a), which seems to reflect the lower incidence of dengue viruses. Previous mosquito infection rates with dengue virus at a time of low transmission in Puerto Rico yielded similarly low values (0.098 – 0.670) to the ones observed in this study (Barrera et al. 2019a). Notably, the pool minimum infection rates of *Ae. aegypti* in this and previous studies were not significantly different among treatments. Since we detect the presence of arboviruses in pools rather than in individual mosquitoes, and we assume that there is at least one positive mosquito in each positive pool, we may be grossly underestimating the true prevalence. This is especially true if the actual infection rate is high, as multiple mosquitoes within a pool may carry the virus. The use of the Vector Index helps better understand the risk of being bitten by an infected mosquito as it represents the expected number of infected mosquitoes per thousand, resulting from the product of the minimum or pool infection rate times the relative abundance of the mosquito. In the previous studies referenced before, Vector Index values during the chikungunya and Zika virus outbreaks were larger (2.4 – 4.3) but somewhat smaller for dengue (0.07 – 0.21) than those in the present study (0.3 – 2.01) (Barrera et al. 2019a).

The *Ae. aegypti* density threshold for dengue reported in this study cannot be generalized to other geographies where key drivers of thresholds such as temperature, herd immunity, rate of virus introductions, and protection against mosquito bites vary. However, values reported here may be helpful as starting values in other tropical areas with similar conditions to Puerto Rico. The present study has several strengths, such as its long duration, multi-arm randomized cluster design, and the inclusion of both entomological and serological outcomes. Further studies should evaluate thresholds under higher dengue transmission conditions or in regions with different climates. It would also be important to evaluate the cost-effectiveness of mass-trapping strategies relative to other control measures. Analyzing the cost of mass trapping should consider several factors: the potential for locally sourcing traps, which could reduce the cost per unit compared to purchasing commercial versions; the

feasibility of improving trap designs to make them easier for residents to handle; and the possibility of involving the community in servicing the traps, which could help lower labor expenses.

Advantages of validated density thresholds include: 1- Vector Control Programs would have well-defined targets for vector management (below or above threshold density); 2- testing the effectiveness of new vector management tools for preventing arbovirus infections is simplified because we would only need to demonstrate whether the vector population was brought below the target density threshold or not (no need for control clusters without treatment); and 3- there is no need to evaluate disease impact, which is costly, because we would have a correlate or proxy for disease prevention.

## Conclusions

This study successfully demonstrated the effectiveness of mass AGO trapping in significantly reducing *Ae. aegypti* populations in urban communities of Caguas City, Puerto Rico. By decreasing mosquito densities to levels below four females per trap per week, we identified a potential threshold associated with reduced dengue virus presence in mosquitoes and minimal increases in dengue antibodies among young residents. Although low dengue transmission during the study limited definitive conclusions about the density-transmission relationship, these findings provide a valuable target for mosquito management programs. Establishing and maintaining such thresholds could enhance vector control strategies and support arbovirus prevention efforts in similar settings.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data availability

Data will be made available upon request.

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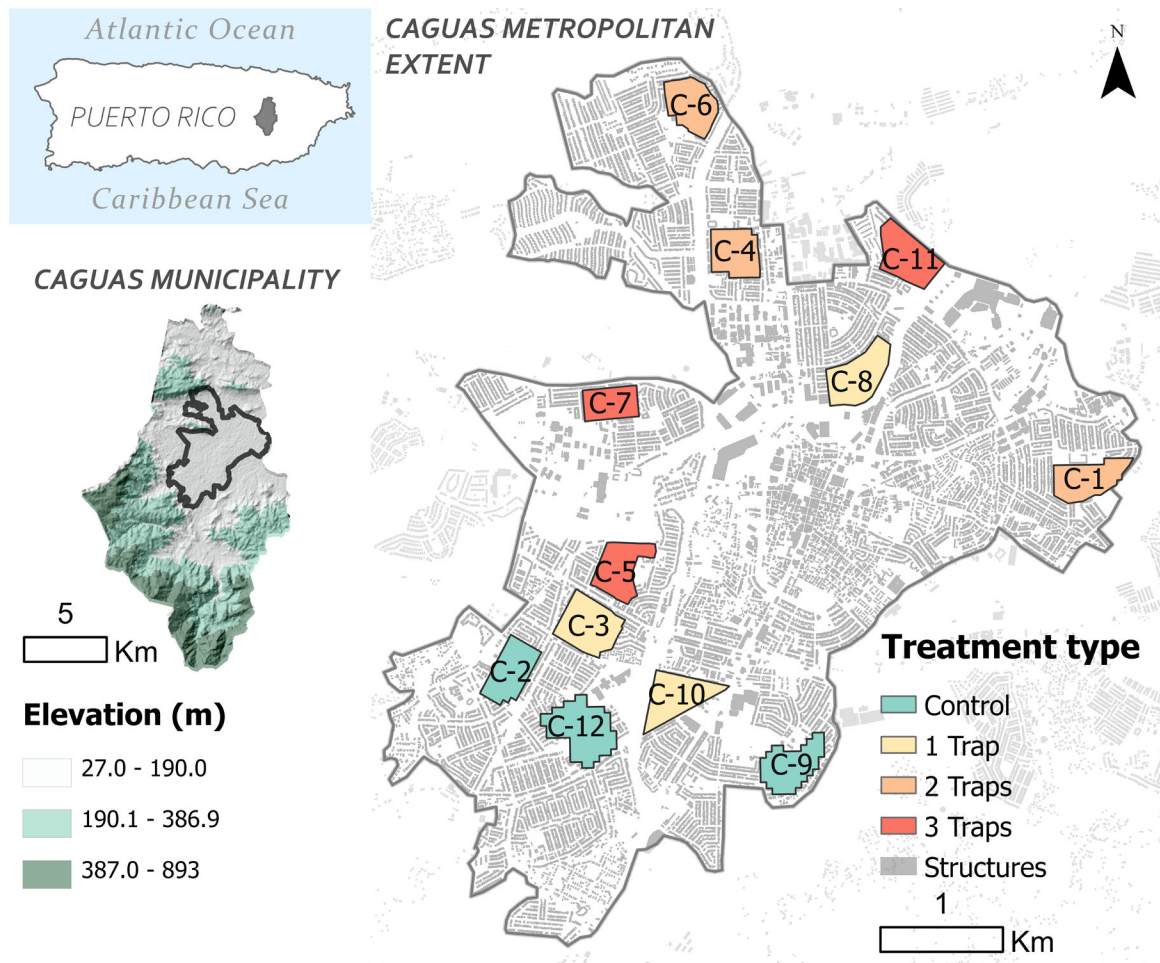


**Figure 1.**

BW: Map of the study area showing the twelve randomly selected study clusters and the type of mass trapping treatment (control, 1, 2, and 3 traps/house) in Caguas city, Puerto Rico.

Color: Map of the study area showing the twelve randomly selected study clusters and the type of mass trap treatment (control, 1, 2, and 3 traps/house) in Caguas city, Puerto Rico.

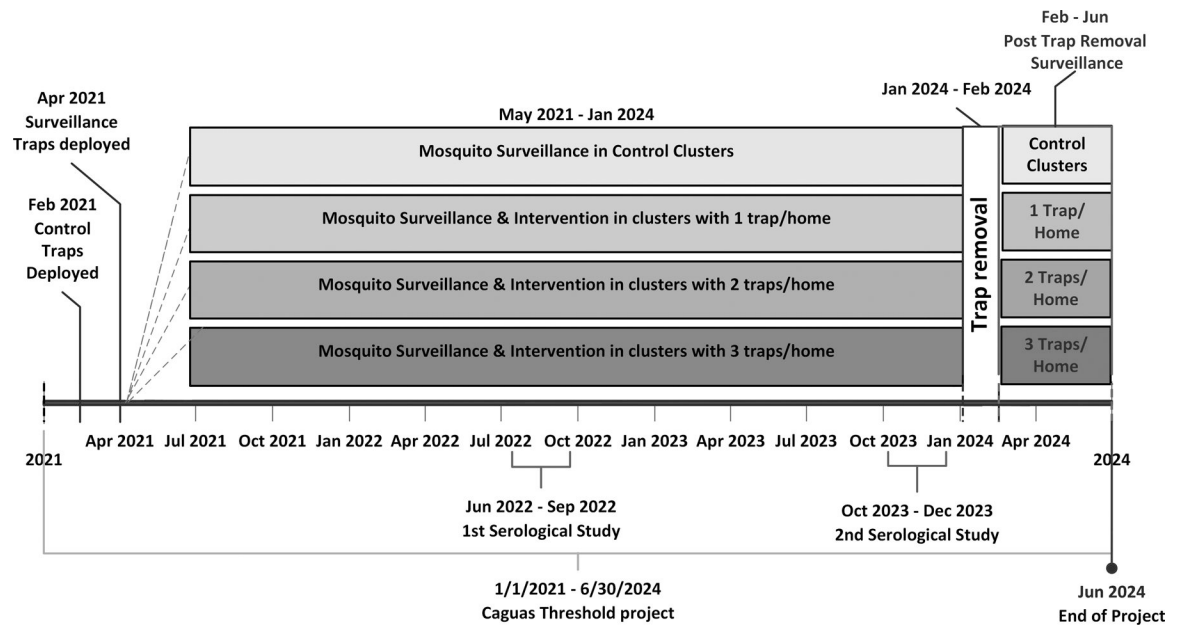


**Figure 2.**

Timelines and activities performed in the four-arm cluster randomized study from January 2021 through June 2024 in Caguas city, Puerto Rico.

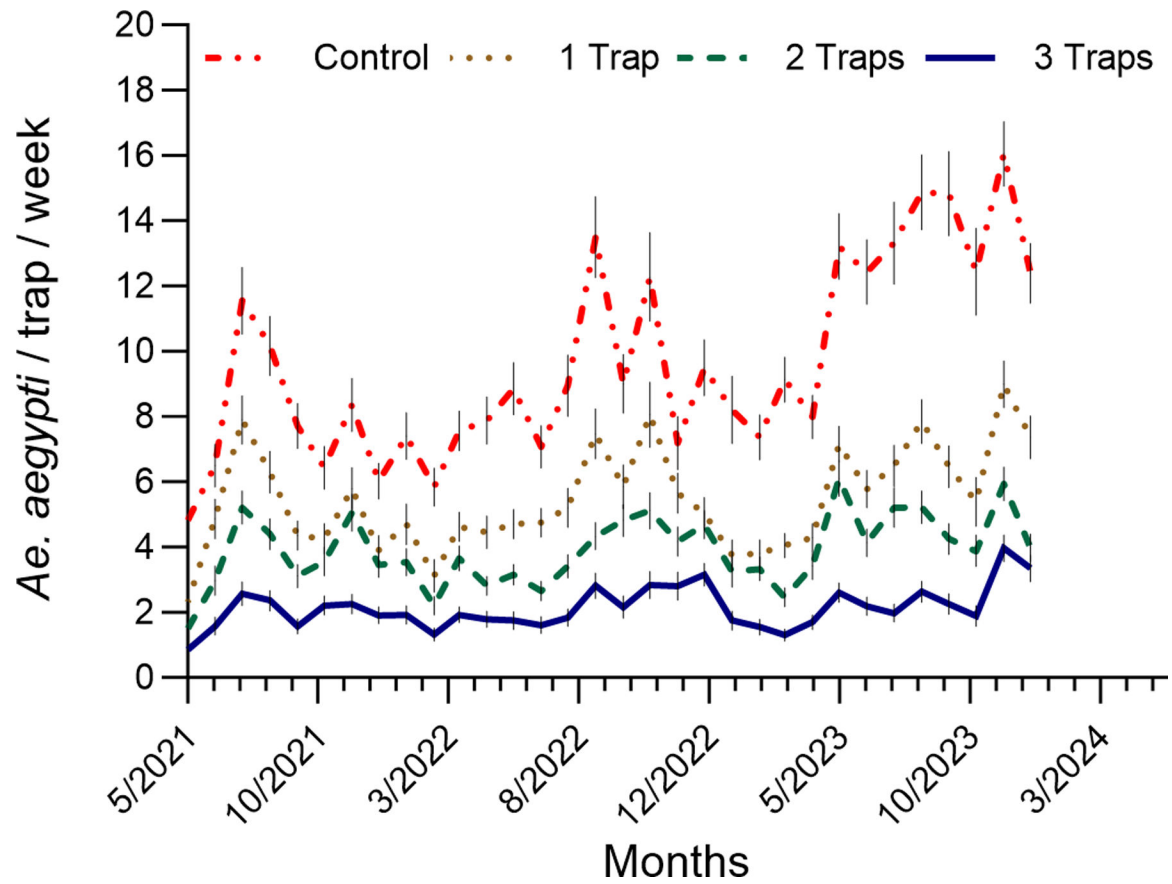


**Figure 3.** Monthly changes in average and 95% C.I. of *Ae. aegypti* females captured per trap per house in the four mass-trapping treatments (control, 1, 2, and 3 traps/house) from May 2021 to December 2023 in Caguas city, Puerto Rico.

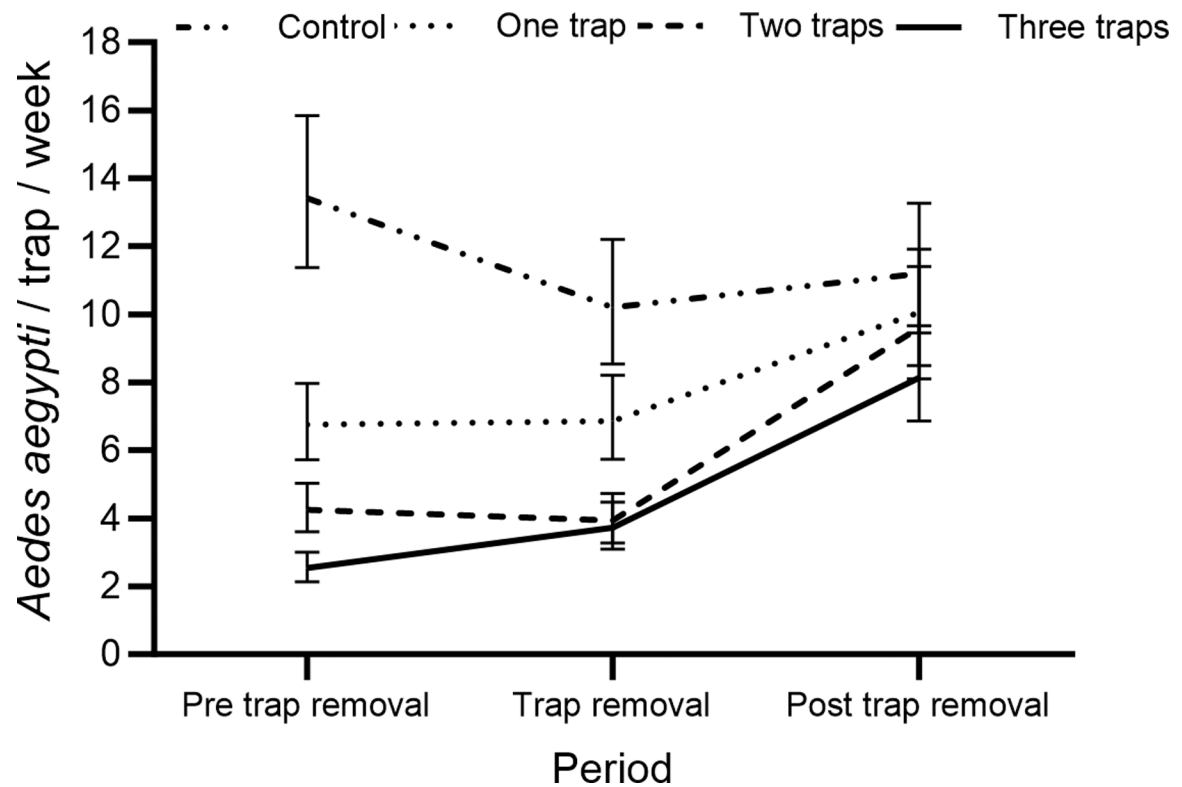


**Figure 4.**

Interactions plot of average and 95% C.I. of *Ae. aegypti* females captured per trap per week in clusters with 0 (control), 1, 2, and 3 traps/house during periods of pre-trap removal, trap removal, and post-trap removal. Data for trap removal periods were from September-December 2023 (pre trap removal), January-February 2024 (trap removal), and March-June 2024 (post trap removal).

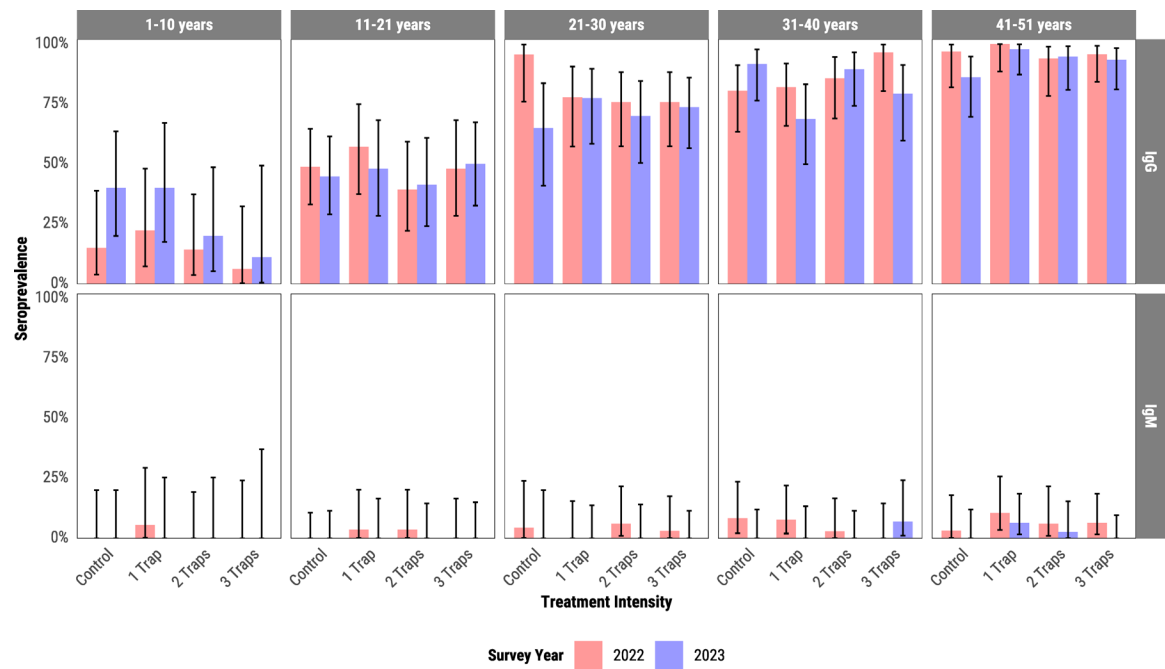


**Figure 5.** IgG and IgM seroprevalence (%) in residents by age groups and treatments for the first (2022) and second (2023) serosurveys conducted in Caguas city, Puerto Rico.

**Table 1:**

Results of the Generalized Linear Mixed Model comparing average numbers of *Aedes aegypti* per trap per week across treatments (# traps/house) and weather variables observed during the study from May 2021 to December 2023 in Caguas city, Puerto Rico.

| Variable                             | Coefficient | Std. Error | Z value | Sig.   | Incidence Rate Ratio (IRR) | 95% Confidence Interval (IRR) |        |
|--------------------------------------|-------------|------------|---------|--------|----------------------------|-------------------------------|--------|
|                                      |             |            |         |        |                            | Lower                         | Upper  |
| Intercept                            | -0.196      | 0.2377     | -0.823  | 0.411  | 0.822                      | 0.5161                        | 1.3102 |
| Control (no IAGO <sup>a</sup> traps) | --          | --         | --      | --     | --                         | --                            | --     |
| 1 trap/house                         | -0.573      | 0.1558     | -3.68   | <0.001 | 0.564                      | 0.4154                        | 0.7651 |
| 2 traps/house                        | -0.925      | 0.1559     | -5.94   | <0.001 | 0.397                      | 0.2921                        | 0.5382 |
| 3 traps/house                        | -1.588      | 0.1560     | -10.18  | <0.001 | 0.204                      | 0.1505                        | 0.2774 |
| Rainfall (mm)                        | 0.002       | 0.0001     | 25.91   | <0.001 | 1.002                      | 1.0017                        | 1.0020 |
| Temperature (°C)                     | 0.059       | 0.0046     | 12.85   | <0.001 | 1.061                      | 1.0513                        | 1.0703 |
| Relative Humidity (%)                | 0.008       | 0.0020     | 3.77    | <0.001 | 1.008                      | 1.0036                        | 1.0117 |

<sup>a</sup>IAGO traps = Intervention AGO traps.

**Table 2.**

Generalized Linear Mixed Model estimated means and 95% Confidence Intervals of *Ae. aegypti* females per trap per week by treatment (control, one, two, and three traps/house) and trap removal period. Data for trap removal periods were from September-December 2023 (pre trap removal), January-February 2024 (trap removal), and March-June 2024 (post trap removal).

| Treatment                            | Period            | Estimated means | 95% Confidence Interval |       |
|--------------------------------------|-------------------|-----------------|-------------------------|-------|
|                                      |                   |                 | Lower                   | Upper |
| Control (no IAGO <sup>a</sup> traps) | Pre trap Removal  | 13.42           | 11.37                   | 15.84 |
|                                      | Trap Removal      | 10.21           | 8.54                    | 12.20 |
|                                      | Post Trap Removal | 11.20           | 9.46                    | 13.27 |
| 1 trap/house                         | Pre trap Removal  | 6.75            | 5.72                    | 7.97  |
|                                      | Trap Removal      | 6.86            | 5.73                    | 8.21  |
|                                      | Post Trap Removal | 10.05           | 8.49                    | 11.91 |
| 2 traps/house                        | Pre trap Removal  | 4.25            | 3.60                    | 5.03  |
|                                      | Trap Removal      | 3.93            | 3.27                    | 4.73  |
|                                      | Post Trap Removal | 9.61            | 8.11                    | 11.4  |
| 3 traps/house                        | Pre trap Removal  | 2.54            | 2.14                    | 3.00  |
|                                      | Trap Removal      | 3.72            | 3.09                    | 4.48  |
|                                      | Post Trap Removal | 8.14            | 6.86                    | 9.66  |

<sup>a</sup>IAGO traps = Intervention AGO traps.

**Table 3.**

Pool infection rates with dengue virus of *Ae. aegypti* females captured in SAGO traps and Vector Index by treatment from May 2021 to December 2023 in Caguas City, Puerto Rico. Differences in pool infection rates between treatments were calculated using the infection rate of the control clusters as the reference.

| Treatment                            | Total Pools Analyzed | Positive Pools | Analyzed Mosquitoes | Average Mosquito Density | Pool Infection Rate<br>(95% C.I.) <sup>a</sup> | Pool Infection Rate Differences<br>(95% C.I.) <sup>a</sup> | Vector Index <sup>a</sup><br>(95% C.I.) <sup>c</sup> |
|--------------------------------------|----------------------|----------------|---------------------|--------------------------|------------------------------------------------|------------------------------------------------------------|------------------------------------------------------|
| Control (no IAGO <sup>b</sup> traps) | 8771                 | 17             | 79943               | 9.68                     | 0.213<br>(0.128,0.333)                         | --                                                         | 2.10<br>(1.013–2.918)                                |
| 1 trap/house                         | 7583                 | 14             | 45256               | 5.48                     | 0.31<br>(0.177,0.507)                          | –0.097<br>(–0.287,0.088)                                   | 1.70<br>(0.850–2.651)                                |
| 2 traps/house                        | 7188                 | 5              | 32409               | 3.92                     | 0.154<br>(0.057,0.341)                         | 0.059<br>(–0.144,0.231)                                    | 0.60<br>(0.121–1.204)                                |
| 3 traps/house                        | 5825                 | 3              | 17738               | 2.15                     | 0.169<br>(0.044,0.457)                         | 0.044<br>(–0.253,0.231)                                    | 0.36<br>(0.000 0.846)                                |
| Total                                | 29367                | 39             | 175346              | 5.97                     | 0.223<br>(0.161,0.301)                         | --                                                         | 1.33<br>(0.922–1.765)                                |

<sup>a</sup> Scale is per 1,000 female mosquitoes.

<sup>b</sup> IAGO traps = Intervention AGO traps.

<sup>c</sup> 95%CI calculated using bootstrap