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Citywide control of *Aedes aegypti* during the 2016 Zika epidemic by integrating community awareness, education, source reduction, larvicides, and mass mosquito trapping.

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Abstract

This investigation was initiated to control *Aedes aegypti* and Zika virus transmission in Caguas City, Puerto Rico during the 2016 epidemic using Integrated Vector Management (IVM), which included community awareness and education, source reduction, larviciding, and mass-trapping with Autocidal Gravid Ovitraps (AGO). The epidemic peaked in August - October 2016 and waned after April 2017. There was a pre-intervention period in October / November 2016 and IVM lasted until August 2017. The area under treatment (23.1 Km^2) had 61,511 inhabitants and 25,363 buildings. The city was divided into eight even clusters and treated following a cluster randomized stepped-wedge design. We analyzed pools of female *Ae. aegypti* adults for RNA detection of dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses using 360 surveillance AGO traps every week. Rainfall, temperature, and relative humidity were monitored in each cluster. Mosquito density significantly changed (Generalized Linear Mixed Model; $F_{8,\ 14588}$= 296; P< 0.001) from 8.0 ± 0.1 females per trap per week before the intervention to 2.1 ± 0.04 after the percentage of buildings treated with traps was 60% and to 1.4 ± 0.04 when coverage was above 80%. Out of a total 12,081 mosquito pools there were one DENV, seven CHIKV, and 49 ZIKV positive pools from October 2016 to March 2017. Afterwards, we found only one positive pool of DENV in July 2017. This investigation demonstrated that it was possible to scale up effective *Ae. aegypti* control to a medium-size city through IVM that included mass trapping of gravid *Ae. aegypti* females.

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Keywords

Mosquito control; *Aedes aegypti*; Zika virus; Vector-Borne Pathogens; AGO traps; Integrated Vector Control

Dengue is the most common arboviral disease of humans in the world and is caused by infections with several flaviviruses (DENV; Gubler 1988). Dengue is present in at least 100 countries in the Americas, Africa, Asia, and Caribbean and Pacific nations (CDC 2018) where it has been estimated to infect as many as 390 million people annually (WHO 2012, Bhatt et al. 2013). More recently, chikungunya (CHIKV) and Zika (ZIKV) viruses, which are transmitted by *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), have spread following regional dengue's spatial distribution (Musso et al. 2015, Bisanzio et al. 2018). Concurrent circulation and coinfection of people with DENVs, CHIKV, and ZIKV have already been reported in the Americas (Carrillo-Hernandez et al. 2018). The prospect for other arboviruses to also spread from enzootic areas into urbanized areas (Weaver, 2018), calls for improving the control of the main urban *Aedes* mosquito vectors.

The last major epidemics of DENV (DENV-1 and −4) in Puerto Rico were in 2010, 2012, and 2013 with 21,298, 12,877, and 18,164 reported cases, respectively (PAHO 2018). DENV epidemic activity has not been detected since. DENV had been detected in *Ae. aegypti*, which is the vector of this virus in Puerto Rico (CDC, unpublished). Autochthonous transmission of CHIKV was detected for the first time in Puerto Rico in May 2014. CHIKV rapidly spread throughout the island in the following months, causing 28,327 reported cases (Sharp et al. 2016). CHIKV virus was detected in *Ae. aegypti* mosquitoes around confirmed cases and in mosquito surveillance sites (Barrera et al. 2017, Felix et al. 2018). Thus far, no further epidemics of this virus have been reported in Puerto Rico. Local transmission of ZIKV was recorded by the end of November 2015 and this virus caused a major epidemic in 2016 with 36,326 confirmed cases (PAHO 2018). ZIKV was detected in *Ae. aegypti* around confirmed cases and in mosquito surveillance sites (Barrera et al. 2018b, Felix et al. 2018), which confirms the involvement of *Ae. aegypti* in the transmission of DENV, CHIKV, and ZIKV in Puerto Rico. Another potential vector of these arboviruses (Pool-Smith et al. 2015), *Aedes mediovittatus* (Coquillett) has not been found infected with DENV, CHIKV or ZIKV in Puerto Rico thus far (CDC, unpublished; Felix et al. 2018). Because Zika infections are associated with developmental defects in infants, substantial resources were mobilized to protect pregnant women from becoming infected and to activate *Ae. aegypti* control (Frieden et al. 2016). This large-scale intervention was initiated as part of the response to the 2016 Zika epidemic in Puerto Rico. The 2016 Zika epidemic in Puerto Rico peaked in August, with over 7,000 new cases per month through middle October, and sharply decreased afterwards through the cooler and drier months that correspond with the boreal winter (December 2016 – April 2017). New Zika cases were less than 100 per month after April 2017 (PRDH 2018).

Traditional *Ae. aegypti* control focuses on visiting houses to manage or eliminate containers harboring immature mosquitoes, apply larvicides, or spraying adulticides (WHO 2009). These approaches are limiting because they require household visits, which result in low vector control coverage as many residents are absent at the time of the visits or some refuse treatment (Chadee 1988) or because cryptic containers indoors and outdoors escape inspection and control (Barrera 2015). Low vector control coverage means that the fraction of the treated *Ae. aegypti* population is not high enough to prevent or control local arbovirus transmission or to cause noticeable reductions of the adult mosquito population (Lounibos 2003, Barrera 2015).

Recent approaches to controlling *Ae. aegypti* are based on the release of conspecific, modified male mosquitoes to suppress the reproductive potential of natural populations and reduce mosquito density using *Wolbachia* bacteria, radiation, or lethal genes (Flores and O'Neill 2018). These methods target the adult mosquito population, apply the concept of area-wide population suppression, and avoid the laborious, traditional vector control. These emerging techniques are being evaluated in open field investigations to understand if the approaches can improve disease prevention or control, and have yet to be used within the framework of an integrated vector control approach.

An alternative strategy to controlling *Ae. aegypti* is by mass trapping adult female mosquitoes (Johnson et al. 2017). Successful control of *Ae. aegypti* with mass trapping has been achieved in small human communities (Barrera et al. 2014a, Barrera et al. 2014b). Traps targeting gravid, ovipositing females reduce both the number of biting mosquitoes and their reproductive potential. Successful use of gravid traps requires having an efficient trap, enough traps per home or area, sufficient coverage or houses with traps, and timely servicing (Johnson et al. 2017, WHO 2018). A previous study in Puerto Rico using Autocidal Gravid Ovitraps (AGO traps) showed 50% lower prevalence of CHIKV antibodies in residents of communities with three AGO traps per home in the yards of most houses (Lorenzi et al. 2016). Average mosquito densities associated with such protection by mass trapping in that study were lower than two-three female *Ae. aegypti* per AGO trap per week (Barrera et al. 2017). Keeping the female *Ae. aegypti* population below this level was also associated with significantly lower incidence of CHIKV and ZIKV in field-collected mosquitoes during 2014 and 2016, when these viruses invaded Puerto Rico (Barrera et al. 2018b). Previous studies comparing trap captures of *Ae. aegypti* in Puerto Rico showed that less than three females per AGO trap per week roughly corresponds to one female per modified Sentinel BG-trap per day and three eggs per ovitrap per day (Barrera et al. 2014b). Identifying a mosquito density associated with human protection is important to inform when effective vector control has been achieved. Low mosquito densities that are partially protective against infections with newly introduced arboviruses such as CHIKV and ZIKV for which there was no human herd immunity will also likely be protective against endemic DENVs. The reason is that the human population has varying levels of partial protection due to previous infections; and the number of mosquitoes required to cause a local dengue outbreak is expected to be larger in human populations with higher levels of immunity against the circulating virus (Focks et al. 2000).

This public health intervention applied Integrated Vector Management (IVM), consisting in community awareness, education, source reduction, larviciding, and mass mosquito trapping to: 1- reduce the transmission of DENVs, CHIKV, and ZIKV as indicated by the detection of viruses in local *Ae. aegypti* populations, 2- determine if the level of *Ae. aegypti* reduction previously observed in smaller communities using mass trapping could be replicated at the scale of a medium-size city; that is, reducing the density of *Ae. aegypti* below the putative two-three females per trap per week threshold, 3- better define the percentage of vector control coverage that is required to effectively reduce the *Ae. aegypti* populations below this level, and 4- learn what limitations might arise when scaling up this type of vector control intervention. Because this work was conducted during the 2016 Zika epidemic in Puerto Rico, we treated all areas of the city in a random, sequential order using a Cluster Randomized Stepped Wedge (CRSW) approach.

Materials and Methods

Intervention area.

The IVM intervention took place in Caguas City, Caguas Municipality (18.23412 N, −66.0485 W). The population and number of buildings in the municipality were 142,893 (United States Census Bureau 2010) and 65,852 (Municipal Revenue Collection Center 1998), respectively. The treated area (23.1 Km^2) included 61,511 inhabitants and 25,391 buildings. We developed a Geographical Information System (GIS) to monitor progress and produce maps with layers of roads (Puerto Rico Highways and Transportation Authority), buildings (Municipal Revenue Collection Center), satellite images (DigitalGlobe™; June 25, 2014), location of mosquito surveillance traps and mosquito abundance, houses treated or untreated (abandoned, no response, refused treatment), and type of treatment. The city was divided into eight clusters with similar numbers of buildings and the order of IVM treatment was selected at random (Figs. 1, 2; Table 1). A flowchart summarizes the study (Fig. 2).

Ethics approval and consent to participate.

The proposal was presented, discussed, and approved by municipal and state health authorities, and a written agreement stating the scope and length of the intervention was signed by all parties. The Centers for Disease Control and Prevention determined that this intervention did not involve human subjects under 45 CFR 46.102(f). We explained to adult residents participating in the intervention the nature of the project and asked for their written permission to conduct IVM on their properties, return every two months to service the AGO traps, and in some of them return every week to count the number of mosquitoes in surveillance traps. Residents could opt out at any time.

Community awareness.

We conducted six focus groups with 50 community leaders representing residents of 37 communities in the City of Caguas to explain the project and learn about residents' acceptance of the use of mosquito traps in their communities, including installing three mosquito traps per home (CDC 2017). We further reached out to the residents in the city by conducting town hall meetings involving 154 community leaders from 150 communities to discuss how to protect against Zika infections and our projected application of IVM methods, including education, container removal, larvicides, and mass trapping with AGO traps. We established an agreement with the Government of Puerto Rico's Call Center (311) to provide information to concerned residents and to orient them who to contact for further information.

Vector surveillance.

We began monitoring adult female *Ae. aegypti* mosquitoes in October 2016; six weeks before the initiation of vector control. We deployed 360 fixed-position surveillance AGO traps (SAGO traps) that were monitored every week throughout the study (Fig. 1). AGO traps attract and capture gravid *Ae. aegypti* females on a sticky surface located inside a 3.8 liters, black plastic capture chamber that is partially inserted into a 19 liters black plastic bucket containing 10 liters of water and a 30 grams hay packet (Barrera et al. 2014b). Mosquitoes were counted in these surveillance traps by a team of eight technicians every week under CDC's supervision. We did not monitor mosquitoes in the AGO traps that were used for intervention purposes (IAGO traps. 3 per structure). When a house had one SAGO trap then only two IAGO traps were placed in that house. SAGO traps were serviced every two months to replace water, sticky surface, and hay. SAGO traps (38–53 per cluster) were deployed to uniformly cover the eight clusters, avoiding areas that were 100 m from the edge of neighborhoods, highways, fields, industrial complexes, and streams. Distances between SAGO traps were 109 – 130m. Each SAGO trap's geographical coordinate was used as a centroid to produce one Tiessen polygon per trap covering the entire intervention area, so that analyses could be performed at both cluster and polygon (SAGO trap) levels. A Thiessen polygon is drawn so that each SAGO trap is at the center of each polygon and the borders of polygons define areas that are the closest to each SAGO trap's center considering all neighboring SAGO traps. Each of the 360 Tiessen polygons was used to aggregate data at that level (e.g., number of IVM-treated and untreated structures around a given SAGO trap).

Virus surveillance in adult mosquitoes.

Females of *Ae. aegypti* collected from every SAGO trap every week were transported to the laboratory where they were pooled by trap (1– 20 specimens per pool) and stored at −80ºC until processing by a trioplex RT-PCR to detect RNA of DENV, CHIKV, and ZIKV (Santiago et al. 2018). RNA of these viruses can be detected in *Ae. aegypti* mosquitoes exposed in the field for more than one week (reviewed in Barrera et al. 2018b).

Integrated Vector Management (IVM).

Vector control activities were contracted to a pest control company (Rentokil/Oliver Exterminators). Vector control started on November 14, 2016 and the project ended on August 4, 2017, when contractors began removing control traps. Trap removal and subsequent follow up was interrupted by Hurricane Irma on September 6, 2017 and then by Hurricane Maria on September 20, 2017. IVM consisted of distributing educational handouts to adult residents, source reduction (removing only small disposable containers), applying larvicides (Four Star, *Bacillus thuringiensis israelensis* CRG) to containers with water not used for pet or human consumption, and setting three IAGO traps or more per building, depending on the size of the property (residential house, commercial store, etc.). Traps were acquired from SpringStar Inc. (Woodinville, WA) at $14.50 each.

We distributed the following handouts to households in the study area: notification that residents were to be visited by authorized and identified personnel from a specific pest control company to conduct IVM in their properties, illustrated explanation about the use of IAGO traps and actions residents can take to eliminate *Ae. aegypti* mosquitoes in their premises, and notifications that residents were to be visited to service the IAGO traps and repeat IVM. If mosquito larvae were detected in containers when visiting properties every two – three months to service the IAGO traps, the containers were either removed (small ones), treated with larvicides, or the resident was advised to cover or protect the container. In large public areas such as shopping centers, industries, and parks IAGO traps were placed at least 50m apart from each other. The initial goal of the intervention was to treat 80% or more buildings in each of the eight clusters because such a treatment coverage achieved significant and sustained control of *Ae. aegypti* in previous studies (Barrera et al. 2014a, b). We expected to gain insight at this scale about the minimum coverage required to bring down the mosquito population below two-three females per trap per week.

Hired personnel were instructed on the transmission of viruses by *Ae. aegypti*, its biology and ecology, how to monitor and control this mosquito, filling data forms, using maps to orient themselves in the field, and use of portable electronic devices to enter data (Table S1). Additional training sessions were provided given the high turnover of personnel throughout the project. The number of field personnel conducting vector control fluctuated between 34 and 100 per month. More personnel were required as the study progressed to service the traps that had already been deployed, besides adding traps to yet untreated structures. Vector control personnel were asked to visit each structure up to three times on different times and days, including weekends, to be able to contact the residents.

We gathered the following data: name of resident, address, geographical coordinates, telephone number, date of visits, unique structure identifier, treatment cluster, status of premise (no response, rejected treatment, treated, abandoned, vacant), types of containers treated or managed (covered, placed under a roof) to prevent mosquito production, number and identifier of each IAGO trap installed, and if larvicide was used. Data were collected using a password protected, encrypted software application that was developed in-house and was capable of running on cell phones, tablets, and personal computers. Data were stored and managed in a CDC server.

Estimating the number of mosquitoes removed from the environment by control traps.

The number of adult *Ae. aegypti* mosquitoes eliminated by mass trapping during the study was calculated by multiplying the average number of mosquitoes captured in SAGO traps by the number of deployed IAGO traps per week. This method is based on an investigation carried out in Villodas, Guayama Municipality, Puerto Rico from December 2016 through April 2017, where we found no significant differences between the numbers of female *Ae. aegypti* mosquitoes captured per trap per week in 27 SAGO traps (1 per house) and in 60 randomly selected IAGO traps out of a total 570 IAGO traps deployed (three per house; CDC unpublished). Characteristics of the Villodas study site are published (Barrera et al., 2014b).

Weather.

Because clusters were treated at different times and because *Ae. aegypti* populations are mainly driven by rainfall and relative humidity in Puerto Rico (Lega et al. 2017), we used meteorological parameters as covariates in all analyses. We placed and operated meteorological stations (HOBO Data Loggers, Onset Computer Corporation, Boume, MA) in the center of each of the eight clusters, where daily temperature, relative humidity, and rainfall were recorded throughout the intervention. To relate weather variables to mosquito abundance, we averaged daily temperature and relative humidity for three weeks before sampling and accumulated rainfall during the third and second weeks before sampling. Assuming a mosquito life span of three weeks, the number of adult *Ae. aegypti* mosquitoes captured in the traps may have been influenced by temperature and relative humidity during the previous three weeks. Accumulated rainfall during the week before sampling is not expected to modify the number of adult mosquitoes in the traps as immature development lasts about a week.

Statistical analyses.

We tested the hypothesis that the average number of mosquitoes captured in SAGO traps significantly decreased as the percentage of treated houses increased. We used a Generalized Linear Mixed Model (GLMM) analysis on the number of female *Ae. aegypti* per trap per week with percentage of houses treated in the vicinity of each trap (Thiessen polygon) as the main factor and meteorological parameters as covariates. Clusters (1–8) were used as random factors (Hemming et al. 2015). We used a negative binomial model with log link for all analyses because the variance of the count variable was greater than the mean, and a first-order autoregressive function as the covariance structure of the repeated measures. We repeated this analysis using an interval-transformed variable of the percentage of houses treated, with the following values: 0, 1–20, 21–40, 41–60, 61–80, and > 80% that represent the progress in treatment coverage in time. We were particularly interested in investigating what would be the minimum treatment coverage necessary to cause a steady, significant reduction of the *Ae. aegypti* population. A posteriori mean comparisons were analyzed using adjusted sequential Bonferroni tests at a significance level of 0.05.

We contrasted the density of *Ae. aegypti* before and after treatment for each of the clusters using Generalized Estimating Equations (GEE). We considered that a cluster had been minimally treated at the time when the percentage of treated buildings reached 60%. This resulted from the observation that the overall density of *Ae. aegypti* stabilized around two females per trap per week when treatment coverage exceeded 60%, as reported below. We considered a transition time of three weeks for the *Ae. aegypti* population to respond to treatment (Fig. 3), as observed before (Barrera et al. 2018a). Data collected during the transition time was not included in the analyses.

We used a GEE for a citywide comparison of final and pre-treatment *Ae. aegypti* densities. The pre-treatment period included eight weeks from October 10 – December 5, 2016 (vector control operations slowly started in November 14, 2016) and was compared with an eight-week period before the end of the project, from June 12 to August 4, 2017. Meteorological co-variates were used as before to control for differences in weather between final and pre-treatment periods. Results were reported as means and standard errors. Statistical analyses were carried out using IBM SPSS Statistics 25 software (IBM Corporation, Armonk, NY, USA).

We calculated the maximum likelihood minimum infection rates (MIR) of mosquitoes using PooledInfRate version 4.0 (Biggerstaff 2016). The Vector Index [VI = (N * P)* 1,000], an indicator of the expected number of infected mosquitoes per trap per week, was calculated as the proportion of infected mosquitoes (P = positive pools / total pools tested) times the average number of mosquitoes (N) captured per trap per week per 1000 (Jones et al. 2011).

Results

Community outreach.

Fifty community leaders participated in the focal groups. The leaders were 20–81 years old (average 56 years old) and 58% were females. Most leaders (98%) had consensus that AGO traps would reduce the number of mosquitoes in their homes and were willing to allow the placement of three traps in their yards. They also expressed that risk of infection with Zika would be reduced by using the traps. Community leaders felt that required trap maintenance would be easy. They suggested that community leaders and neighbors would be trusted sources for educating residents about AGO traps and assisting with trap maintenance, although residents were not asked to service the traps. In practice, community leaders were effective facilitators, particularly in some communities that required their presence to allow technical personnel to apply vector control.

Vector control.

Residents of 20,235 structures accepted installing three or more AGO traps, depending on the size of the property. We deployed 78,126 IAGO traps during the intervention with an average of 3.3 ± 0.01 traps per structure omitting structures with more than 10 traps (N= 511) to cover large areas such as cemeteries and parks. Treated structures reflected the predominance of single story houses but several large properties were also treated, such as shopping centers, industries, and cemeteries. Average time to servicing traps and reapplication of source reduction and larviciding when needed was 94.00 ± 0.17 days (median = 82 days). Commonly found containers that required disposal, management (store under a roof, cleaned up, etc.) or larviciding were: water meters, trash cans, cavities in the structure or its surroundings such as concrete or fencing, bromeliads, plastic buckets, flower pots, barrels, and lids on top of buckets or barrels.

Access to treatment varied among clusters, so that the actual sequence in which treatments was completed varied from the initial plan (Fig. 3). An important aspect affecting treatment schedule and slowing down operations was the need to revisit structures at least three times before giving up because of residents' absenteeism. Structures for which we did not receive authorization (absent resident, refusal) were excluded from treatment. The first round of treatment was completed in all clusters by June 2017. Trap coverage (percentage of buildings treated) was 79 – 84% in six clusters (19,323 buildings) and 67% in two clusters (clusters 2 and 4; 6,068 buildings). Clusters 2 and 4 had the smallest percentage of residential properties of all clusters (25 – 37%; Table 1). The number of contractors dealing with IVM and trap servicing increased from 34 –77 during the first months (November – April) to 80 – 100 later because of the increasing number of traps requiring maintenance.

We conducted 15,480 SAGO trap weekly inspections (traps * weeks) from the beginning of the study until August 4, 2017 when trap removal started. We captured 62,760 female *Ae. aegypti* (4.06 ± 0.04 mosquitoes/SAGO trap/week; min, max = 0, 72). Rainfall peaked during late November 2016 and early December 2016, when we observed the largest density of mosquitoes in the SAGO traps (Fig. 4). Rainfall steadily decreased towards March 2017, as is normal during this period in Puerto Rico (Barrera 2010) and mosquito density concomitantly decreased. Mosquito density then stabilized at a density just above two mosquitoes per trap per week when trap coverage reached 60% or above (mid-March – May 2017; 2.10 ± 0.04 mosquitoes/trap/week) in spite of increased rainfall. In subsequent months (June – August 4, 2017) overall mosquito density stabilized below 2 mosquitoes/trap/week when trap coverage was at or above 80% in most clusters (1.4 ± 0.04; Fig. 4).

The GLMM model of the average number of mosquitoes captured in SAGO traps per week, as a function of vector control and weather variables, was significant ($F_{4, 15067}$= 628; P< 0.001). Significant effects were the cumulative percentage of houses that had been treated in the vicinity of the SAGO traps ($F_{1, 15067}$= 779; P< 0.001), temperature during three weeks before sampling ($F_{1, 15067}$= 30; P< 0.001), relative humidity during three weeks before sampling ($F_{1, 15067}$= 150; P< 0.001), and cumulative rainfall registered on the third and second weeks before sampling ($F_{1, 15067}$= 13; P< 0.001). The exponent of the coefficient for the percentage of houses treated (0.986) indicated a 1.4% reduction in the number of mosquitoes per trap per week per unit increase in the percentage of houses treated.

We repeated this analysis using the percentage of houses treated per week as an interval variable (0, 1–20, 21–40, 41–60, 61–80, and >80%) in order to estimate what mosquito reduction effects existed at each level of vector control. The model was significant ($F_{8,14588}$= 296; P< 0.001), with significant effects of the interval-coded percentage of treated houses per week treated in the vicinity of the SAGO traps ($F_{5, 14588}$= 146; P< 0.001), temperature during three weeks before sampling ($F_{1, 14588}$= 8.5; P< 0.01), relative humidity during three weeks before sampling ($F_{1, 14588}$= 163; P< 0.001), and cumulative rainfall registered on the third and second weeks before sampling ($F_{1, 14588}$= 13.2; P< 0.001). We saw no significant mosquito reduction of the average number of mosquitoes when control coverage was 0 – 20%, and significant reductions (α < 0.001) when control coverage increased to 21 – 40% (34.3% reduction), 41 – 60% (42.4%), 61–80% (62%), and >80% (81.5%; Fig. 5). The observed and predicted densities of *Ae. aegypti* per trap per week when 80% or more of the houses had been treated were 1.41 ± 0.05 and 1.68 ± 0.20, respectively.

Each cluster had specific characteristic changes in mosquito density, rainfall, and treatment schedule (Figs. 6, 7) but the general tendency of mosquito density of reaching small and steady values with increasing treatment coverage in each cluster was similar to the overall average tendency (Fig. 4). Clusters 1 and 8 had less rainfall than the other clusters and Cluster 8 had the highest average temperature and lower relative humidity, but in general, weather among clusters was not strikingly different.

The GEE comparisons of *Ae. aegypti* density before and after reaching 60% treatment (allowing a transition period of 21 days for the treatment to take effect) were significant for every cluster (Table 3). The meteorological parameters of relative humidity and rainfall were the covariates more frequently and significantly associated with changes in mosquito density other than vector control. The citywide comparison of final versus initial *Ae. aegypti* densities was significant for vector control treatment ($\chi 2$= 709.0; P< 0.001), rainfall ($\chi 2$= 59.0; P< 0.001), and temperature ($\chi 2$= 16.1; P< 0.001). Average initial and final densities of *Ae. aegypti* were 7.97 ± 0.12 and 1.41 ± 0.04, respectively. This result translates into an effective reduction of the mosquito population of 82.3% {[1- (Final density after treatment / Initial density)] * 100}.

Arbovirus surveillance in female *Ae. aegypti*.

We analyzed 12,081 pools of female *Ae. aegypti* for the presence of virus RNA of DENV, CHIKV, and ZIKV until 4 August 2017. We found 49 positive pools for ZIKV, seven for CHIKV, and two for DENV. The only positive pool found after March 2017 when the treatment coverage was above 60% was one positive pool of DENV in July (Fig. 8). ZIKV was detected in consecutive weeks from the beginning of the study in October 2016 until February 2017. We did not find any ZIKV positive pool in cluster 7, 15 positive pools in Cluster 3, and the rest of clusters had between four and seven positive pools each. During that period ZIKV MIR per week was 1.10 (0 – 2.81) mosquitoes (per 1,000) and VI was 8.87 (0 – 28.12) (per ,1000). The average density of *Ae. aegypti* per trap per week that yielded positive pools for ZIKV was 9.8 ± 0.1 (N= 49; range 1 – 37). CHIKV was detected in low, sporadic frequency from November 2016 to March 2017. Positive pools came from clusters 1, 3, 4, and 7. CHIKV MIR per week was 0.25 (0 – 1.32) mosquitoes (per 1000) and VI was 1.03 (0 – 2.81). Positive pools for CHIKV came from traps with an average density of *Ae. aegypti* of 10.3 ± 3.7 (N=7; range 1 – 29). The two DENV positive pools came from clusters 1 and 8. Positive DENV pools came from traps with 2 and 6 females of *Ae. aegypti*.

Expected number of mosquitoes removed from the environment by control traps.

The total expected number of adult *Ae. aegypti* captured in AGO traps from deployment in November 2016 until August 2017 was 6,223,826. Given that the human population under treatment were 61,511 inhabitants, the expected number of mosquitoes removed per person was around 100 or 10 per person per month. The expected number of adult mosquitoes removed peaked in December 2016 and January 2017, and decreased afterwards. The cumulative number of mosquitoes removed per month steadily increased during the intervention without evidence of leveling off.

Discussion

This intervention evaluation in Caguas was successful in achieving low, steady mosquito density values at several times the scale of previous studies in Salinas, Puerto Rico. The analyses also indicate that when treatment coverage, or percentage of treated buildings, was below 20% no mosquito population reduction was noticeable, and percent reduction increased proportionally to the percentage of treatment coverage above this level. Mosquito populations stabilized at around two mosquitoes per trap per week when coverage was above 60% and less than two when coverage was over 80%. These results suggest that vector control coverage does not need to reach 80% to effectively reduce *Ae. aegypti* density to low and stable levels in this city, which can substantially reduce costs. A recent study using IVM (including mass trapping) and Citizen Action to control biting *Ae. albopictus* similarly showed the importance of achieving significant vector control coverage (Johnson et al. 2018).

One of the objectives of this intervention was to determine if female adult *Ae. aegypti* density in the city could be brought down to a steady 2–3 per trap per week at this geographic scale. This target mosquito density derives from studies conducted in smaller communities since 2011 in Puerto Rico (Barrera et al. 2014a, b, Barrera et al. 2017, 2018a). In those studies, source reduction and larviciding were applied only at the beginning of the study, and mass trapping has continued without any additional vector control actions. When CHIKV s invaded Puerto Rico in 2014, this project was in place and consisted of two communities with three IAGO traps per house in most houses (>80%) and two nearby communities without vector control. Both density of *Ae. aegypti* and CHIKV RNA detection in this mosquito were about ten times higher in the untreated communities and CHIKV antibody prevalence was 50% lower among people from treated communities than from untreated communities after the CHIKV outbreak (Lorenzi et al. 2016, Barrera et al. 2017). When ZIKV invaded Puerto Rico by the end of 2015, we found results similar to those for CHIKV (Barrera et al. 2018b).

We have been using a combination of source reduction and larviciding along with mass trapping following the early recommendations coming from the successful experience of the vector control program of Singapore back in the 1970's (Chan et al. 1977). This investigation did not attempt to isolate individual effects of community outreach, education, source reduction, larviciding, or mass trapping. Rather, we pursued evaluating the impact of this integrated approach. When we consider that some aquatic habitats of *Ae. aegypti* can be super productive, such as a single septic tank found in a house in southern Puerto Rico producing an excess of 1,500 adult *Ae. aegypti* per day (Barrera et al. 2008), then using an integrated vector control approach seems justified. In this intervention, we found a single house that had its first floor inundated with sewage and a large population of immature *Ae. aegypti* that required treatment with larvicide. One can argue that the importance of using integrated vector control approaches is to compensate for deficiencies of individual vector control tools.

The Zika epidemic in Puerto Rico peaked in August - October 2016. This public health intervention was implemented to help reduce the incidence of this disease, but the study could not start before October 2016 because AGO traps were not initially commercially available. A company (SpringStar) had received a Small Business Innovation Research Grant from the National Institutes of Health to produce a commercial prototype for mass production, but the Zika epidemic occurred before the company was in the position to mass-produce traps. The results of this intervention showed that not just ZIKV was circulating in Caguas but also DENV and CHIKV, as these viruses were detected in pools of female *Ae. aegypti*. The frequency and continuousness of ZIKV detections in locally collected mosquitoes indicated ongoing transmission from October 2016 at the beginning of the study through March 2017. The presence of these arboviruses in *Ae. aegypti* is an indication of local transmission, because this mosquito species does not fly very far; virus detections in local mosquitoes can thus be used as a xenosurveillance tool (Grubaugh et al. 2015). CHIKV virus was less frequently detected and not in consecutive weeks, and DENV was detected in only two occasions. Arboviruses in mosquitoes mostly disappeared after March 2017 (until July) when vector control coverage was above 60% and average *Ae. aegypti* density was around two.

Unfortunately, because the study was initiated so late into the epidemic, we cannot make definitive conclusions about the impact of this IVM intervention on transmission because Zika cases waned throughout Puerto Rico after April 2017 (PAHO 2018). Previous dengue and chikungunya epidemics dissipated, like Zika, after December (Barrera 2010, Sharp et al. 2016), possibly as a combination of saturation of hosts (when most infective bites go into already immune persons) and decreased temperature.

It is unlikely that reduced transmission during the boreal winter in Puerto Rico (December – March) results from sharp decreases in *Ae. aegypti* populations. Although the population of *Ae. aegypti* tends to decrease at that time because of drier conditions, its numbers persist sufficiently high to sustain arbovirus transmission albeit lower levels. Because of it, DENVs have been endemic in Puerto Rico for decades (Barrera 2010). An important factor why *Ae. aegypti* populations do not crash during the drier seasons is that people keep containers with water (Barrera et al. 2011).

Interest is increasing in developing new vector control tools and in testing their impact on epidemiological outcomes (Achee et al. 2015) using appropriate field experimental designs, such as parallel cluster randomized controlled trials (PCRT; Wilson et al. 2015). The main reason for conducting PCRTs is having appropriate, contemporaneous untreated areas to compare with treated ones. Yet, purposely leaving areas untreated to increase the validity of this study was not an option because of the patent risk of Zika infections. This study also underscores another challenge to demonstrating significant reductions in arbovirus infections or disease by vector control: the transitory nature of urban arboviral outbreaks. Any vector control efficacy trial should run long enough to capture the moment when emerging or re-emerging arboviruses invade the experimental areas.

We note that the Zika epidemic in Puerto Rico occurred the year following the onset of a “super El Niño” (Chen et al. 2017), facilitating an early epidemic peak (August), much like the major previous dengue epidemics in Puerto Rico and other Caribbean nations (CDC, unpublished; Amarakoom et al. 2008). El Niño usually brings hotter and drier conditions to Puerto Rico, opposite to the cooler and wetter effects of La Niña (Malmgren et al. 1998). La Niña followed El Niño between July and September 2016, and La Niña lasted intermittently until February – March 2018 (http://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php). Thus, the rapid disappearance of Zika cases during the winter 2016/2017 may have been facilitated by the cooling brought about by this La Niña climatic event.

Successful vector control may be achieved by a combination of activities including: using an efficient vector control agent, applying an effective dosage of the control agent, having a thorough and efficient delivery system to treat most of the area occupied by the vector, reaching sufficiently high coverage to impact a significant proportion of the vector population in an area-wide fashion, reapplying the control agent at proper time intervals, involving communities so that people welcome and cooperate with the types of vector control interventions used, and implementing an integrated vector control approach. The AGO trap has a relatively high efficiency of >83% daily capture of gravid *Ae. aegypti* females in large outdoor cages (CDC, unpublished). We have previously demonstrated that three traps per home can be effective, without much gain by using a fourth one (Mackay et al. 2013). This evaluation suggests that treatment coverage above 60% along with source reduction and larviciding at the time of servicing the AGO traps can bring the mosquito density to levels consistent with disease control. Treatment reapplication, in particular trap maintenance was variable in this intervention, but apparently sufficient to prevent mosquito populations from bouncing back. We involved the communities at the beginning of the study and determined that the proposed type of vector control was acceptable. Although we did not evaluate the relative importance and contribution of source reduction, larviciding and mass trapping to mosquito density reductions, we believe that these control tools could have complemented each other. Another important aspect of vector control that we did not evaluate here was sustainability, which has to do not only with effectiveness but also with funding and costs. This intervention was a vertical or top-down approach, with contracted personnel executing vector control. Community members were involved mostly in allowing the field personnel to gain access to their properties. A next step would be to explore the possibility of having community members participate along a Vector Control Program in a top-down / bottom-up partnership, in which the vector control program would supply advice, monitoring, and needed resources for residents themselves to apply vector control in a sustained way. In such an approach, people would be involved in conducting clean-up campaigns and servicing the traps every two-three months. A similar approach has recently shown effectiveness at controlling *Ae. albopictus* (Johnson et al. 2018).

Another important objective of this evaluation was to learn what limitations might arise when scaling-up this type of vector control intervention. This intervention was planned to be a CRSW, such that we would sequentially treat all areas of the city during the Zika epidemic without leaving areas untreated. Because previous evidence indicated that the intervention has a protective effect, leaving areas untreated could have left residents in those areas at higher risk of Zika infection. We found out that the rate at which vector control could be applied varied between clusters because of the local composition of buildings and land use that determined whether residents were available or willing to participate. We anticipated the need for 100 – 110 field technicians to treat all eight clusters within eight weeks, but hiring and training such number of personnel was not possible. For these reasons, completing treatments was slower than planned. We also learned that contract field personnel needed more training in the use of maps and electronic devices to capture data. The field personnel hired to carry out vector control had no experience working for a Vector Control Program nor previous entomologic training.

In conclusion, the public health intervention described here to control an ongoing Zika epidemic showed entomological impact, by reducing the density of *Ae. aegypti* to levels that were protective against CHIKV infection in a previous, smaller scale study of mass AGO trapping (Lorenzi et al. 2016). We proposed a hypothetical threshold of female *Ae. aegypti* density of two-three specimens per AGO trap per week (Barrera et al. 2017). The results from this investigation showed that such a threshold can be attained in a middle-size city such as Caguas. We also used the presence of arboviruses in *Ae. aegypti* as an indicator of local transmission and the results showed its potential usefulness, although the relationship between incidence of arboviruses in *Ae. aegypti* and people merits further investigations. Finding proxy indicators of human infection without the need for bleeding people could simplify the evaluation of vector control measures and the effectiveness of emerging vector control tools.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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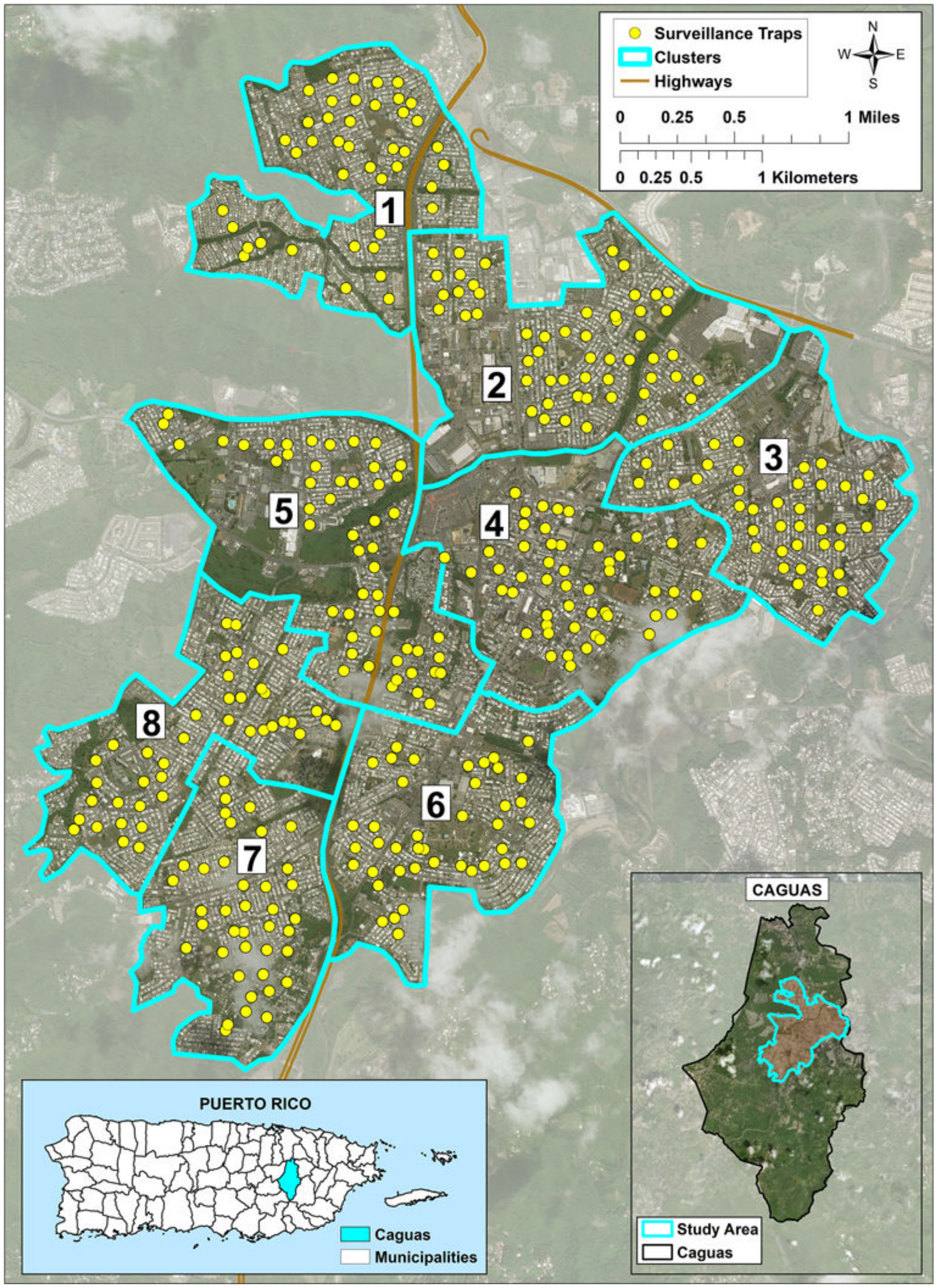

Figure 1.

Map showing a satellite image and geographical location of Caguas City, Caguas Municipality, Puerto Rico. The map highlights the eight zones or clusters in which we divided the city so that each one had similar numbers of buildings. The map also shows the location of the 360 stationary surveillance AGO traps that were sampled every week during the period of study (October 2016 – August 2017).

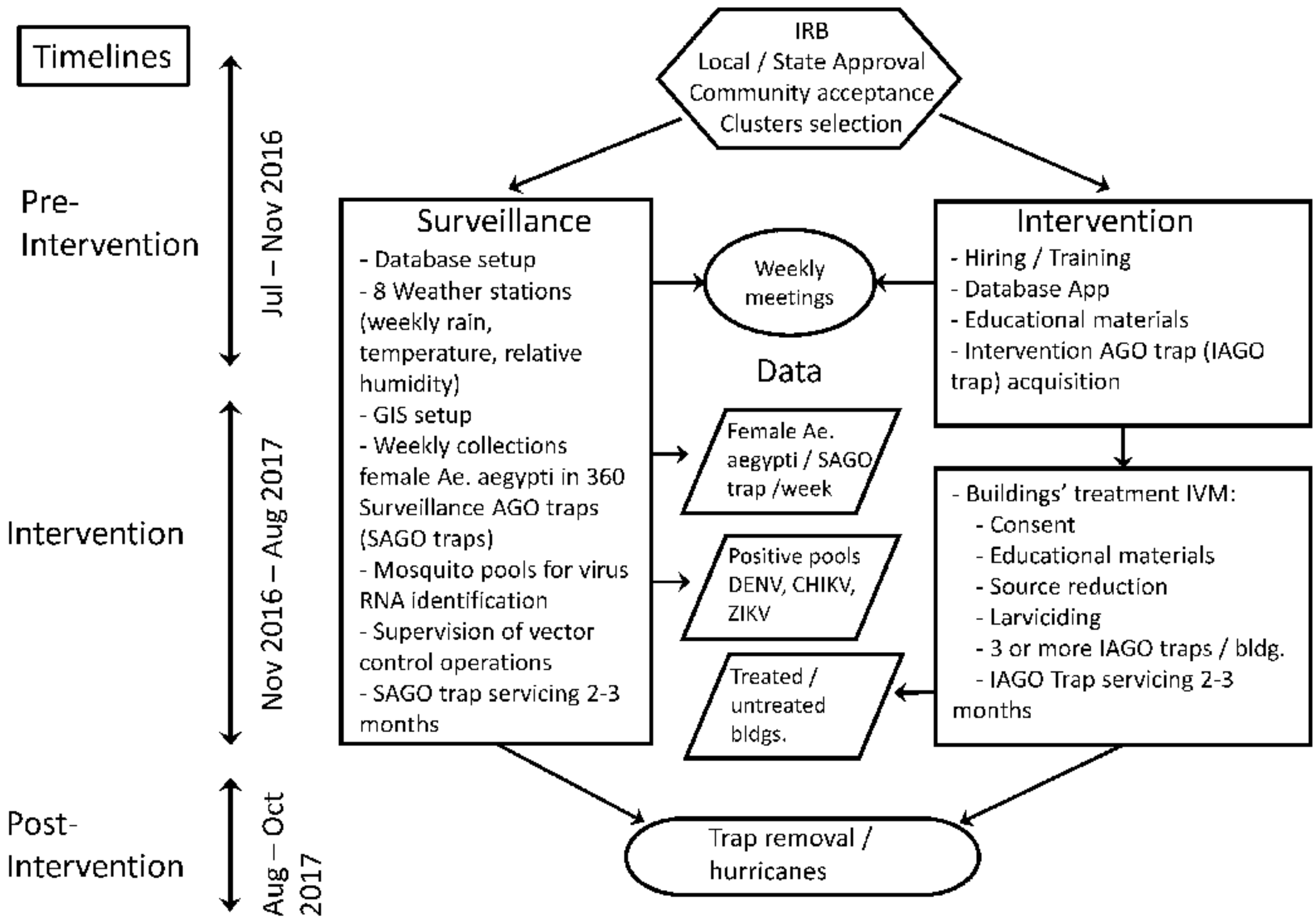

Figure 2.

Flowchart of the study summarizing pre-intervention, intervention, and post-intervention timelines, steps, and results. IRB = Institutional Review Board; AGO= Autocidal Gravid Ovitrap; IVM= Integrated Vector Management.

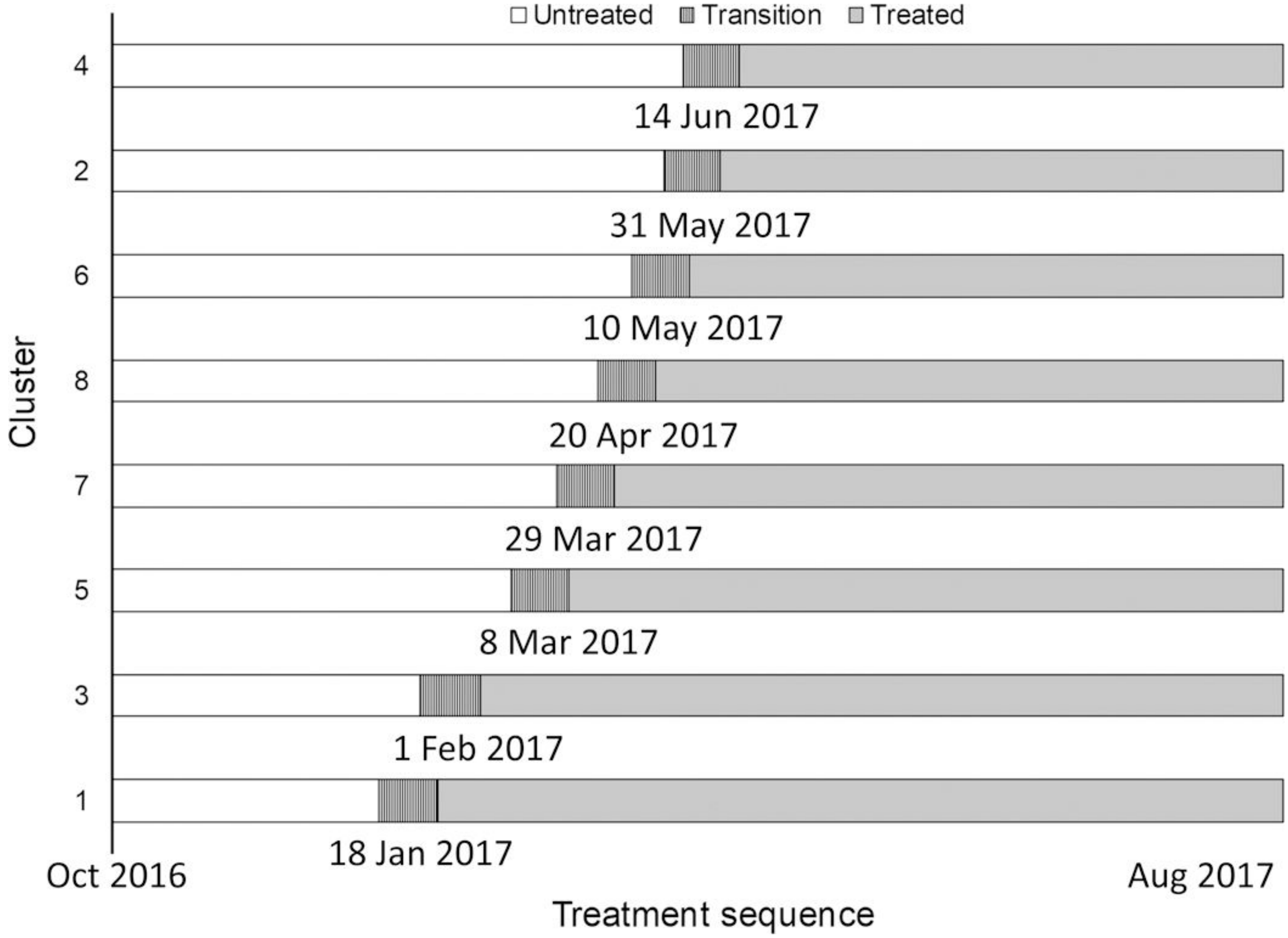

Figure 3.

Order and sequence (steps) of integrated vector control treatments in the eight clusters from October 2016 to August 2017 in Caguas City, Puerto Rico. Dates show when 60% of each cluster had been treated.

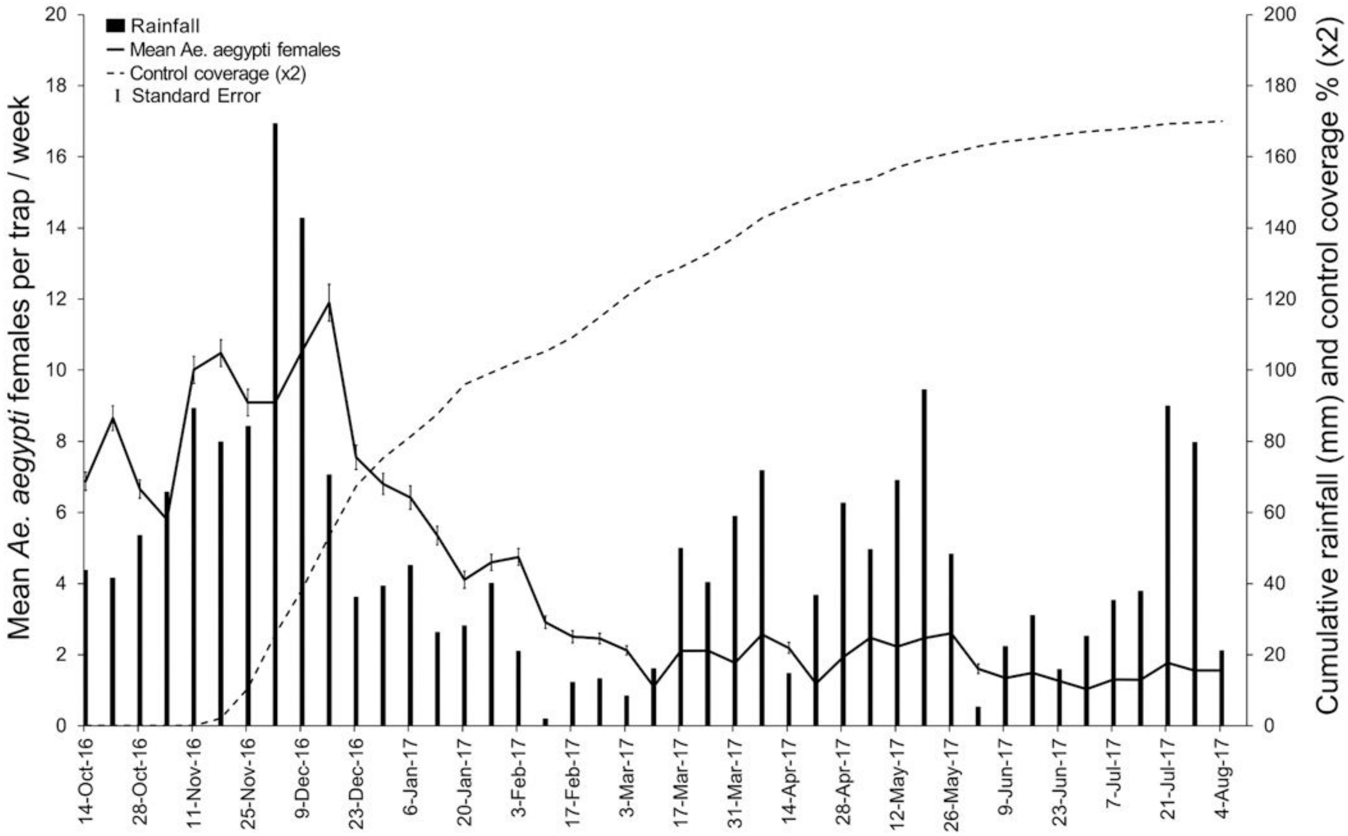

Figure 4.

Weekly changes in the number of female *Aedes aegypti* per surveillance AGO trap, accumulated rainfall 2–3 weeks before sampling, and cumulative percentage of buildings treated with integrated vector control in Caguas City, Puerto Rico (October 2016 – August 2017). Rainfall bars are lagged forward 1.5 weeks to facilitate visual comparisons with the number of mosquitoes. The scale of control coverage has been doubled for presentation purposes.

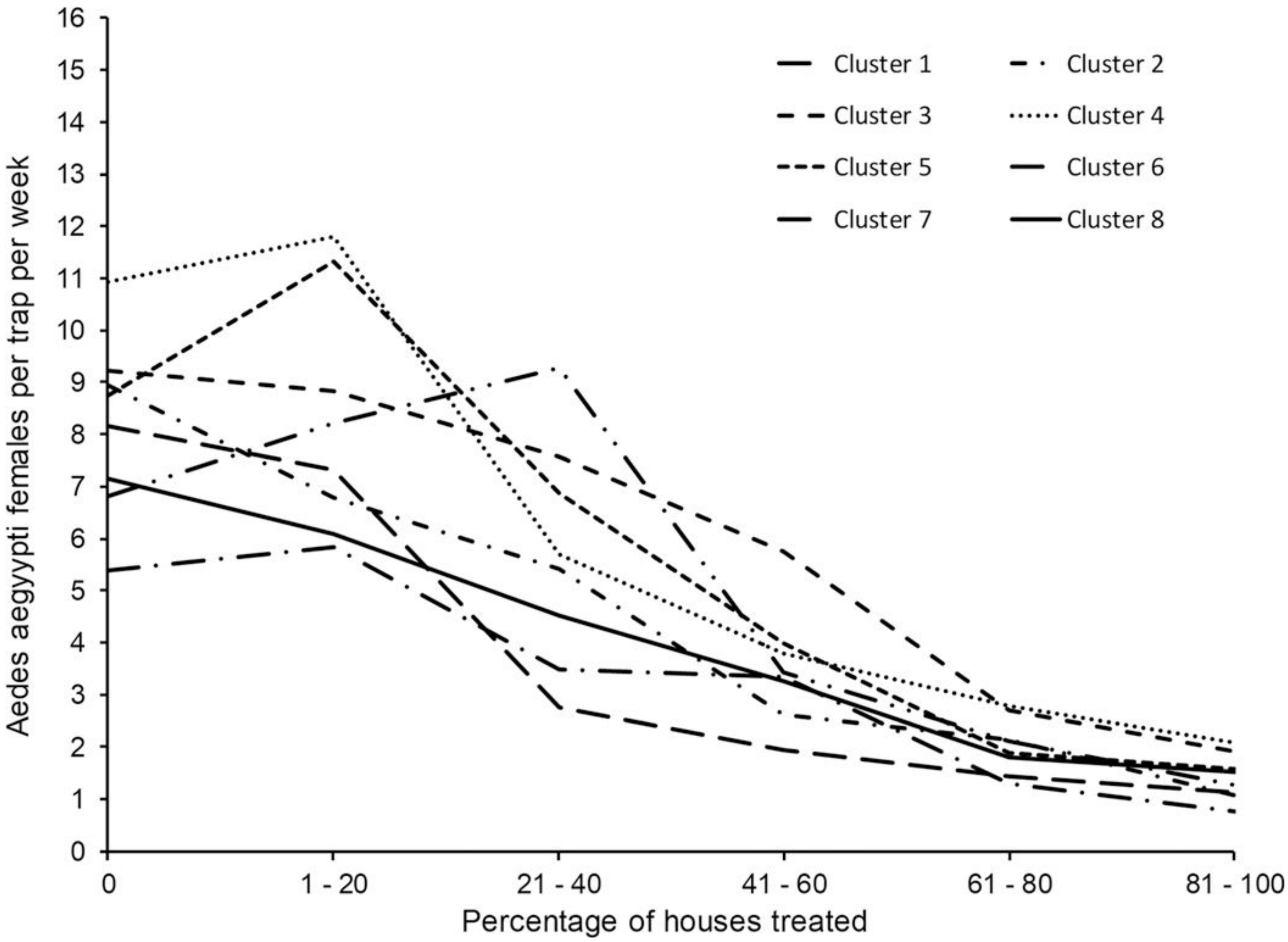

Figure 5.

Change in numbers of *Aedes aegypti* females per trap per week as a function of the percentage of buildings treated with integrated vector control in Caguas City, Puerto Rico.

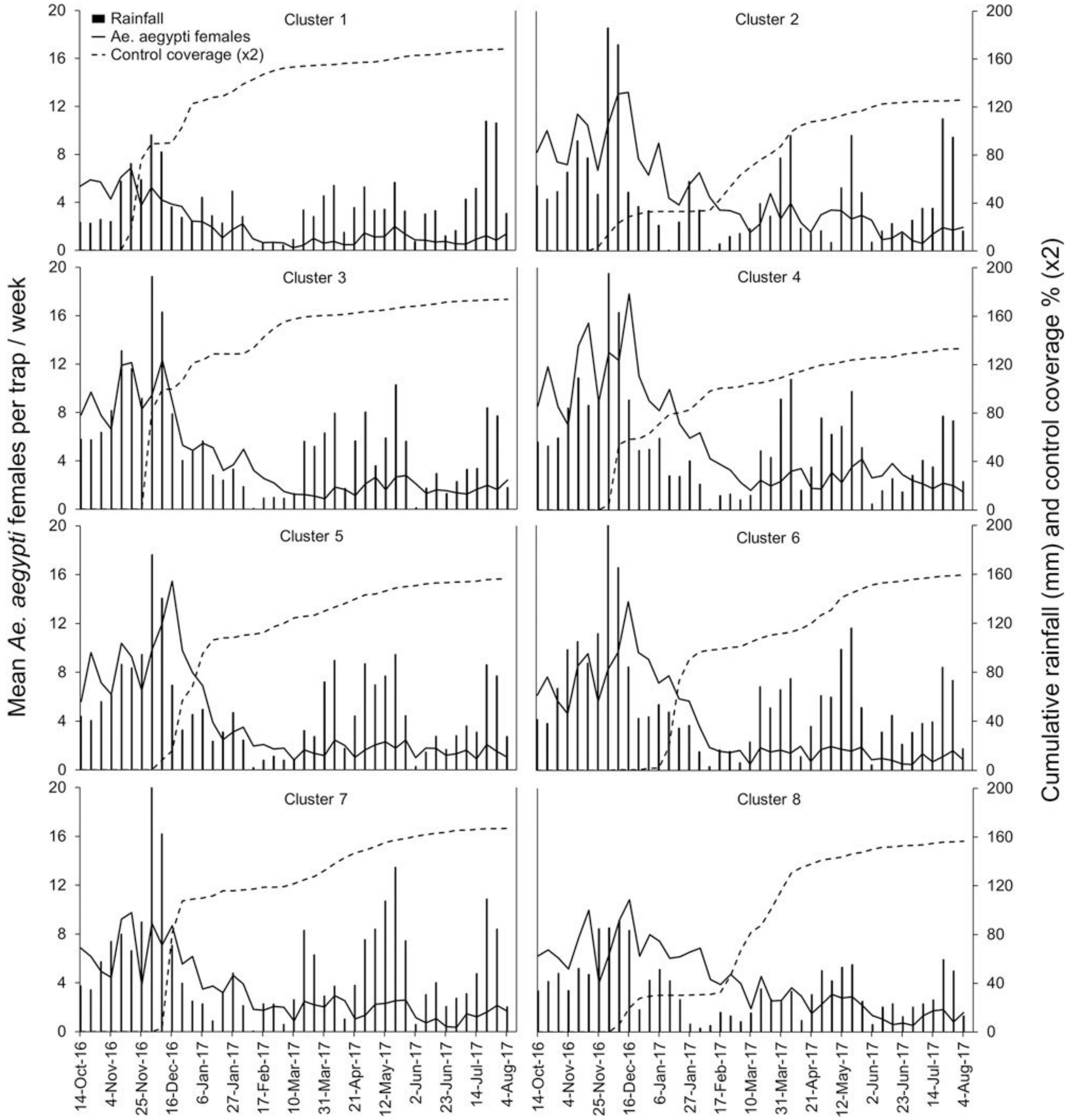

Figure 6.

Variation in the number of female *Aedes aegypti* per surveillance AGO trap, accumulated rainfall 2–3 weeks before sampling, and cumulative percentage of buildings treated with integrated vector control in each of the eight clusters in Caguas City, Puerto Rico (October 2016 – August 2017). Rainfall bars are lagged forward 1.5 weeks to facilitate visual comparisons with the number of mosquitoes.

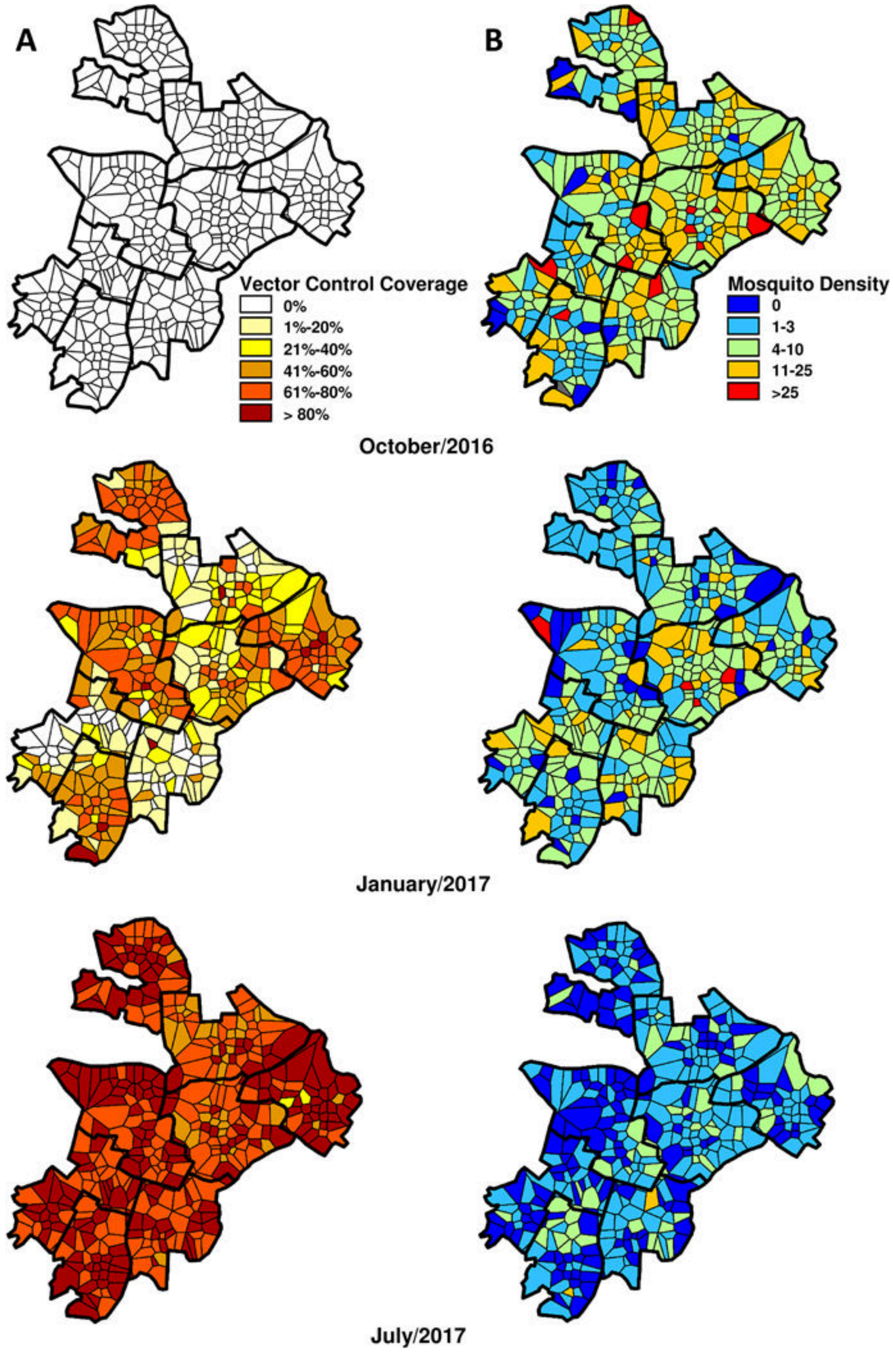

Figure 7.

Thiessen polygons around each of the 360 SAGO traps displaying the percentage of houses treated with IVM (A) and average number of *Aedes aegypti* females per trap per week (B) for three dates from October 2016 to August 2017 in Caguas City, Puerto Rico.

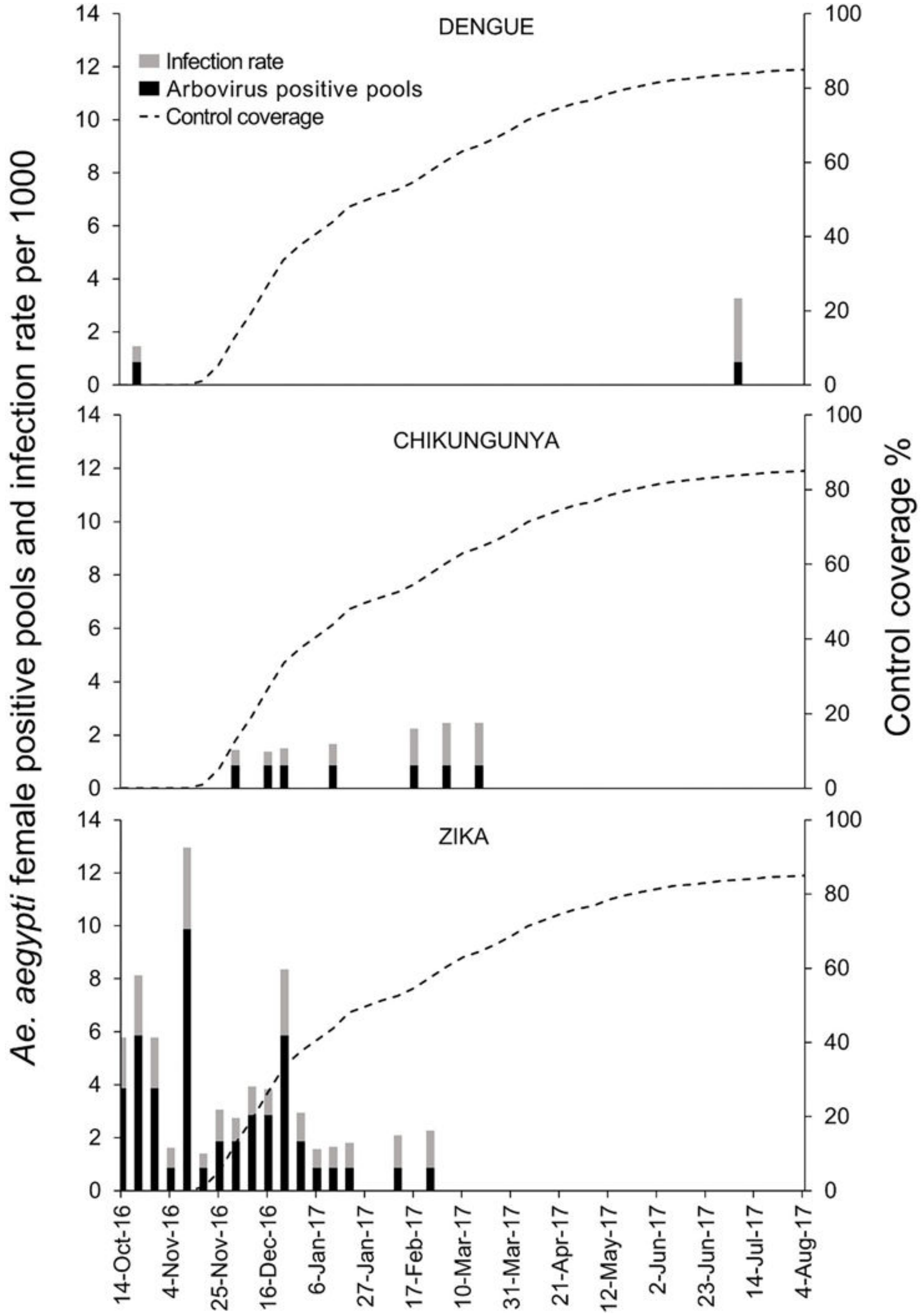

Figure 8.

Number of RNA-positive mosquito pools for Zika, chikungunya, or dengue viruses, infection rates (mosquitoes per 1,000), and percentage of buildings treated with Integrated Vector Control per week in Caguas City, Puerto Rico from October 2016 to August 2017.

Table 1.

Description of the eight clusters in Caguas City where Integrated Vector Management was applied, showing the area of the cluster (km^2), population size (inhabitants), number of buildings, income per household in U.S. dollars, and percentage of land use area: housing units (residential), shops (commercial), mixed housing units and shops, and industrial. Bare soil (e.g., roads), parks and natural areas, sports facilities, and bodies of water occupied the rest of the area not shown in the table, adding to 100%.

Cluster	Area (km^2)	Population	Buildings	Average household income (US $)	% Land use area			
					Residential	Commercial	Mixed Residential / Commercial	Industrial
1	2.72	7,544	3,122	59,000	62.10	0.85	0.22	0.88
2	3.38	10,671	3,086	33,000	37.25	19.13	0.04	10.03
3	2.65	10,094	3,274	30,000	42.12	9.28	0.18	14.94
4	2.47	7,989	2,982	19,000	25.83	17.97	15.59	1.75
5	2.63	11,736	3,523	31,000	40.96	6.17	0.11	10.97
6	2.62	10,548	3,139	29,000	45.59	13.45	0.09	0
7	3.15	12,305	3,182	32,000	62.16	3.57	0.04	1.39
8	3.47	11,268	3,083	37,000	53.74	8.31	0.10	0

Table 2.

Average number of female *Ae. aegypti* in SAGO traps per week (± standard error, sum) and weather parameters from meteorological stations placed at the center of each cluster in Caguas City, Puerto Rico (October 10, 2016 – August 4, 2017).

Zone	SAGO traps	Distance between SAGO traps (m)	Weekly female *Ae. aegypti* / trap ± SE & (Sum)	Weekly rainfall ± SE	Air temperature ± SE	Relative humidity (%) ± SE
1	42	129	2.07 ± 0.08 (3730)	18.60 ± 0.41	25.61 ± 0.04	82.69 ± 0.08
2	53	120	4.78 ± 0.12 (10888)	22.32 ± 0.54	25.87 ± 0.03	80.89 ± 0.08
3	41	131	4.06 ± 0.12 (7156)	26.14 ± 0.65	25.96 ± 0.04	81.42 ± 0.08
4	49	109	5.72 ± 0.16 (11981)	26.77 ± 0.58	25.98 ± 0.03	81.15 ± 0.08
5	54	118	4.02 ± 0.12 (9325)	24.74 ± 0.50	25.82 ± 0.03	81.79 ± 0.07
6	41	116	3.76 ± 0.0.11 (6598)	28.94 ± 0.64	25.62 ± 0.04	83.00 ± 0.08
7	39	125	3.37 ± 0.0.10 (5643)	27.33 ± 0.65	25.53 ± 0.03	82.44 ± 0.09
8	41	111	4.22 ± 0.0.12 (7439)	17.41 ± 0.39	26.11 ± 0.03	75.57 ± 0.10
All	360	120	4.06 ± 0.04 (62760)	24.02 ± 0.20	25.82 ± 0.01	81.13 ± 0.03

Table 3.

Generalized Estimating Equations results (Wald's Chi-Square statistic) comparing the average density (female/trap/week) of *Ae. aegypti* after and before treatment. We compared mosquito densities observed at traps three weeks or more after 60% of the buildings in each cluster (1–8) had received vector control (treated) with densities before that time (untreated). Weekly rainfall, temperature, and relative humidity were covariates to control for temporal change in variables known to influence *Ae. aegypti* dynamics.

Cluster	Vector control (0 ≤ 60% treated; 1 > 60% treated)	Rainfall	Temperature	Relative humidity	*Ae. aegypti* < 60% treated $\bar{X}$ ± STE N	*Ae. aegypti* ≥ 60% treated $\bar{X}$ $\bar{X}$± STE N
1	58.9***	8.6**	0.1 n.s.	0.2 n.s.	4.9 ± 0.2476	0.9 ± 0.11198
2	109,2***	8.3**	11.5**	85.7***	6.1 ± 0.21609	1.4 ± 0.1510
3	49.7***	2.3 n.s.	0.1 n.s.	3.5 n.s.	8.4 ± 0.2550	1.9 ± 0.11089
4	29.9***	0.1 n.s.	3.1 n.s.	62.5***	6.8 ± 0.21583	2.3 ± 0.1364
5	84.6***	7.4**	14.0***	22.5***	7.3 ± 0.2987	1.6 ± 0.11170
6	61.4***	15.1***	1.9 n.s.	257.5***	5.2 ± 0.21124	1.1 ± 0.1511
7	57.3***	4.8*	5.7*	40.1***	5.1 ± 0.2833	1.6 ± 0.1723
8	181.2***	14.1***	1.1 n.s.	10.8***	6.1 ± 0.2998	1.7 ± 0.1640

* α < 0.05;

** α < 0.01;

*** α < 0.001;

n.s. = non-significant

Wald's Chi-Square statistic and significance level (1 degree of freedom) are provided for the vector control factor (0 =before reaching 60% treatment, 1= three weeks or more after reaching 60% treatment), and covariates (weather variables). Average, standard error, and sample size (N= trap x week observations) of female *Ae. aegypti* are shown for each period.