ARTÍCULO ORIGINAL

Evaluation of community-based strategies for *Aedes aegypti* control inside houses

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Introduction: Dengue viruses transmitted principally by the urban mosquito *Aedes aegypti*, cause one of the major public health problems confronting tropical cities. Insecticide spraying has been the mainstay of mosquito control; however, its continuous use has selected for resistance. Other important methods of control involve community participation.

Objective: This study evaluated two control methods for *Ae. aegypti* that can be used by the community: Lethal ovitraps (LOs) and *Bacillus thuringiensis* var *israeliensis* (*Bti*) briquettes.

Materials and methods: The project study was carried out in four similar neighborhoods within a representative district in the city of Cali, Colombia. Three interventions (LO, *Bti*, LO+*Bti* plus education and one control (education only) area were evaluated for efficacy in post-intervention entomological surveys. Additionally, entomological indices were also compared to results from a pre-intervention survey carried out on a sample of city blocks in the same neighborhoods. Relative vector abundance in relation to weather conditions using the same entomological sampling methods was compared.

Results: The interventions did not achieve significant differences in vector abundance among the treatments. However, the interventions achieved a significant reduction in entomological indices compared with those observed during the pre-intervention survey: House index 15.1% vs. 8.5%, mean pupae per house 1.15 vs. 0.073, and Adult index 56.3% vs. 34.8% ($p<0.05$).

Conclusions: The lack of significant differences among the interventions, and between treated and control blocks suggested that educational activities together with periodic visits to the houses produced similar reductions of immature and adult *Aedes aegypti*.

Key words: *Aedes aegypti*, vector control, *Bacillus thuringiensis*, dengue, consumer participation.

Evaluación de estrategias comunitarias para el control de *Aedes aegypti* en Cali, Colombia

Introducción. Los virus del dengue transmitidos principalmente por el mosquito urbano *Aedes aegypti*, causan uno de los mayores problemas de salud pública que confrontan las ciudades tropicales. La aplicación de insecticidas ha sido la base para el control de mosquitos; sin embargo, su continuo uso ha servido para seleccionar individuos resistentes en las poblaciones de mosquitos. Otro método importante para el control involucra la participación comunitaria.

Objetivo. Este estudio evaluó dos métodos de control para *Ae. aegypti* que podrían ser usados por la comunidad: las ovitrampas letales (OL) y las briquetas de *Bacillus thuringiensis* var *israeliensis* (*Bti*).

Materiales y métodos. El estudio se llevó a cabo en cuatro barrios similares de la Comuna 16 de Cali, Colombia. Se evaluaron tres intervenciones (OL, *Bti*, OL y *Bti*) más educación y un área control (sólo educación) para medir la eficacia de la vigilancia entomológica posterior a la intervención. Además, los índices entomológicos se compararon con los resultados de una vigilancia antes de la intervención llevada a cabo en bloques de casas seleccionadas aleatoriamente en los mismos

barrios. La abundancia relativa del vector en relación con las condiciones climáticas se comparó usando los mismos métodos del muestreo entomológico.

Resultados. Las intervenciones no produjeron diferencias significativas entre los tratamientos en la abundancia del vector. Sin embargo, las intervenciones lograron una reducción significativa de los índices entomológicos comparados con los observados en la vigilancia antes de la intervención: índice de casa, de 15,1% a 8,5%; promedio de pupas por casa, de 1,15 a 0,073, e índice de adultos, de 56,3% a 34,8% (p<0,05).

Conclusiones. La ausencia de diferencias significativas entre las intervenciones y el bloque control sugiere que las actividades educacionales junto con las visitas periódicas a las casas producen reducciones similares de los estadios inmaduros y adultos de *Ae. aegypti*.

Palabras clave: *Aedes aegypti*, control vectorial, *Bacillus thuringiensis*, dengue, participación comunitaria.

Dengue virus transmission (serotypes 1-4) by *Aedes* (*Stegomyia*) mosquitoes is a public health problem that principally affects tropical countries. Almost half of the global population lives in high risk areas and currently more than 100 countries experience dengue fever and dengue hemorrhagic fever (1,2). Each year, an estimated 50 to 100 million new cases of dengue occur around the world (3,4); of these, 500,000 cases correspond to dengue hemorrhagic fever with a mortality of 5% (25,000 cases) (1,2,4,5). Due to the lack of a vaccine or an antiviral treatment, disease prevention is based on vector control (1).

The main vector of dengue virus in the Americas is *Aedes aegypti* because of its anthropophilic feeding behavior (6,7); its persistence and resting behavior inside houses (8), and, its capability to inhabit most water holding containers (6,9). Rapid, poorly planned urbanization in association with weak regulatory policies for discharge of solid waste has resulted in the accumulation of discarded containers in most developing countries. These accumulations have favored the establishment and geographic spread of this mosquito. The strategies to control dengue transmission used by the public vector control programs have not been adequate in most countries. The emergence of insecticide

resistance, the difficulty of eliminating larval production through environmental sanitation, and lack of efficacy of ultra-low volume insecticide spraying to control adults are factors which have limited the effectiveness of vector control programs (10,11).

For these reasons, since 1997, in its hemispheric plan to control *Aedes aegypti*, the Pan American Health Organization (PAHO) recognized the need to redirect the control strategy from the exclusive use of chemical control. In particular, PAHO emphasized the need to achieve community participation based on appreciation of the problem together with social communication and education. The community-based strategy requires changes in social conduct that will result in the elimination of mosquito production sites by community action. However, change in behavior and elimination of production sites will be difficult to achieve in the short term. In addition, other vector control strategies are necessary for use by the community that do not use insecticide applications that result in selection pressure for insecticide resistance.

In Colombia, the city of Cali has hyperendemic transmission of all four serotypes of dengue virus (12,13). Here, vector control is based principally on two methods of control. First, immature stages in permanent production sites, such as catch basins (14), are controlled by application of chemical and bacterial larvicides twice a month, and (2) adults are controlled during outbreaks or high infestation levels by use of organophosphate and pyrethroid insecticides (Municipal Secretary of Health, personal communication). Community-based projects are focused mainly on multiunit

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residences and businesses. However, in spite of these vector control strategies, dengue virus transmission persists, and, furthermore, emergence of insecticide resistance has been detected to the organophosphate temephos (15). Additionally, enhancement of mixed-function oxidases and non-specific esterases have been described as two biochemical mechanisms involved in insecticide resistance in these mosquito populations (16,17). The occurrence of insecticide resistance requires a search for alternative strategies for improved vector control.

In the current study four different treatments for *Ae. aegypti* control were evaluated, with community participation and education as additional variables examined. The interventions included (a) the lethal ovitrap, that targets adult gravid females, (b) the application of *Bacillus thurigiensis* var *israelensis* (*Bti*) briquettes, biological larvicide that target larvae stages, (c) use of both treatments simultaneously, and (d) control (no pesticide treatment).

Materials and methods

Rationale

A main objective was to evaluate alternatives to the application of chemical larvicides to containers that were environmentally benign, low in cost and less likely to produce insecticide resistance. The lethal oviposition trap (LO) (U.S. patent № 5,983,557, November 11th, 1999) consists of a black plastic cup fitted with a paper strip that is impregnated with the fast-acting, pyrethroid insecticide, deltamethrin. The LO has been found effective against *Ae. aegypti* in the laboratory (18) and in the field (19). The LO is designed to attract and kill gravid *Ae. aegypti* females (18,19). Females depositing eggs contact the deltamethrin-treated paper strip and obtain a lethal dose of insecticide. Since the LO kills gravid *Ae. aegypti* females, the trap targets the epidemiologically relevant portion of vector populations and thereby decrease transmission of dengue virus. The LO method is environmentally benign, and appears to be a promising strategy to integrate with vector control programs based on community participation. The briquettes

composed of *Bti* control larval stages of *Ae. aegypti* (20). The floating briquette formulation (Bactimos Briquettes, Summit Chemical Co., Baltimore, MD, EPA registration № 6218-47) releases a constant, low dose of bacterial toxins for a period of a month or more, and has been used to control *Ae. aegypti* in its main production sites, water storage containers in houses. *Bti* is a biological insecticide that is environmentally safe and the briquettes can be used by the community after minimal instruction (21).

Study design

The study was continued over a 2-year period from 2004 to 2005. The study was centered in *Comuna 16* (an administrative division for public services that correspond to several neighborhoods) because numerous cases of dengue had occurred there during the previous five years, with an average of 85 cases per 100,000 inhabitants per year. From this *comuna*, four neighborhoods were selected: República de Israel, Mariano Ramos, Unión de Vivienda Popular and Antonio Nariño (figure 1). All of these neighborhoods had similar demographic parameters in terms of property size, population density and socio-economic strata (these were classified as level 2 of 6 social stratification levels, as defined by the Congreso de Colombia. Ley 142 de 1994 (11 de julio), artículo 102). These neighborhoods were representative of more than half of the Cali population with basic public services and houses constructed of bricks and a clay tile roofing. The regularly spaced configuration of city blocks permitted a symmetrical experimental design.

Four treatments were evaluated on four city blocks within each neighborhood: (a) LO, (b) *Bti* briquettes, (c) LO+*Bti*, and (d) control (no pesticide intervention). One block was selected in each neighborhood using random numbers generator; however, the randomly selected blocks were accepted if they had no commercial activity and were located at a minimum of 500 m apart from the previously selected block. Before activating the interventions, all families from the selected blocks were informed about dengue and the study goal (see education activities).

Figure 1. Map of Cali with *Comuna* divisions and the *Comuna 16* map indicating the neighborhoods (limited by a black line) and houses (black dots) sampled during the pre intervention period (2004).

Informed consent was obtained from an adult in the house, who agreed to participate as well as to allow biweekly visits to his/her house. In addition, in the selected blocks, all the water in the containers were discarded explaining to the owner the reason for doing it, and suggesting to keep them dry and clean or dispose them if possible.

After this process, one intervention methodology was randomly assigned to each block, including the block that did not receive any intervention (control block). The control block was expected to serve as a comparison of the effects of education, the initial container removal and the biweekly visits by the project team. The interventions were applied to every house in the selected blocks (approximately, 40 houses per block in an area of 100 m²) and in every house along the treatment block edge; the latter houses were designated as the buffer zone and consisted of approximately 40 houses (10 houses by side) (figure 2). The buffer zone was expected to provide a barrier to re-infestation of *Ae.*

aegypti into the intervention block by eliminating females immigrating from the outside. Once the interventions were initiated, 10 houses in each experimental block were randomly sampled every 15 days to estimate *Ae. aegypti* densities. The interventions were continued for a period of four months (April-July, 2005); this included a high (April-May, 313 mm) and low (June-July, 116.5 mm) precipitation period. The sample size of 10 houses per intervention block was chosen to avoid visiting the same house at regular intervals. Data from each house consisted of establishing the entomological indices (house, pupal and adult) and identifying the mosquito species.

Before the intervention period, weekly entomological surveillance (house, pupal and adult indices) during the previous year (May to October, 2004) was carried out in randomly selected blocks of the Comuna 16, using the same sample size (10 houses) per block. This surveillance permitted an estimation of the entomological indices for the area to see if they were of similar
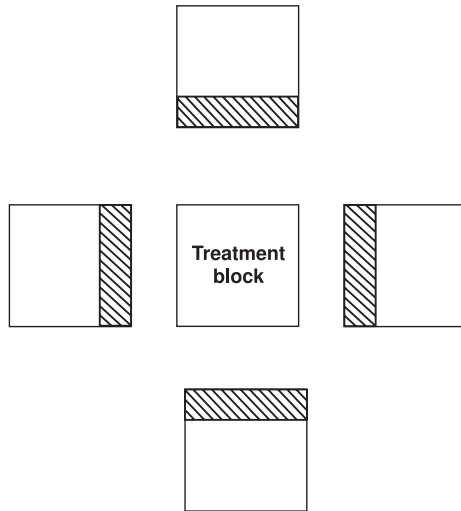
Figure 2. Scheme of the experimental design for each treatment block and treated buffer zones (shaded areas).

magnitude among the neighborhoods, as well as to characterize the types of mosquito-positive containers present. This period also included high and low precipitation periods –April-May (360 mm) and June-July (108 mm). The use of the same sample size during the pre-intervention and intervention periods permitted a comparison of the entomological indices and changes in mosquito densities due to weather changes. Before the intervention, laboratory tests were conducted to measure the lethality of the lethal ovitrap on gravid females and first instar larvae. The tests were conducted for a one month period, using local captured mosquitoes in indoor and outdoor ambient conditions.

Mosquito sampling

The entomological survey was conducted in the selected houses to estimate the abundance of *Ae. aegypti* larvae, pupae and adults. At each site, every water-holding container was inspected. Containers were placed in the following categories: high (rooftop) water storage tanks, low (groundlevel) water storage tanks (washbasins), tires, flower pots or plant in water, bottles, cans, small miscellaneous (<500 ml volume) and large miscellaneous (>500 ml). For each container, the water volume was measured and the presence of larvae and/or pupae was

recorded. Containers with *Aedes* larvae and/or pupae were recorded as positive. All the pupae were collected, counted, packed in separate tubes labeled by site, and transported to the laboratory at CIDEIM for species identification after adult emergence. Larvae were left in the containers during the intervention period to avoid a reduction in the mosquito population. Sampling of adult mosquitoes inside each house was accomplished with modified electric manual aspirators (Insect Vac aspirator, Bioquip) as described by Perich *et al.* (19). Captured mosquitoes from each house were placed in a separate cage and transported to the laboratory for identification.

Entomological data were used to calculate the following indices: house index (percentage of houses infested with larvae and/or pupae); pupal index (mean number of pupae per house); and adult index (percentage of houses infested with adult mosquitoes). Entomological surveys were completed twice a month in identical fashion for all interventions and the control area.

In addition to the household surveys, catch basins were inspected for mosquitoes during the survey and intervention. Catch basins (stormwater collection along streets) are important production sites for mosquitoes in Cali because they hold water permanently (14). The catch basins are cement boxes of 1 m x 0.5 m x 0.8 m of size that maintains an average of 100 to 150 liters of water permanently. Two catch basins, present in the area where the houses were sampled, were evaluated for mosquito larvae. Each catch basin was opened and sampled by taking 20 dip collections with a 300 ml cup and recording the presence of larvae and pupae. All pupae collected in each catch basin were counted and identified after their emergence in the laboratory. During the study, the Secretary of Public Health of Cali separately continued their ongoing control program in the catch basins with the insect growth regular Triflumuron® applied biweekly.

Efficacy of the lethal ovitraps after environmental exposure

Before the intervention, LO efficacy was evaluated under local conditions. Traps were placed in

an empty lot next to the CIDEIM laboratory to simulate the environmental conditions in which the LO would be exposed at the house sites. *Ae. aegypti* Rockefeller susceptible strain and a local strain collected from the study area were used to bioassay the toxicity of LOs that had been exposed to two environmental conditions: inside the house and in an open area (backyard). In the bioassays, the mortality rate of blood fed females three days after feeding and second instars larvae was evaluated after exposure to LOs that had been placed in the two environmental conditions for periods of 10, 20 and 30 days. Each assay consisted of the following tests: (a) three replicate cages with 10 bloodfed females and 10 larvae, each containing a cup with a deltamethrin (0.1 mg) strip, (b) two replicate control cages, each with a cup containing a new strip (positive controls), and (c) a cage without strip (negative control). After 24 hours, survival rates of larvae and adults in each cage were recorded. Additionally, adult mortality was recorded at 45-minute intervals for the first 5-8 hours of each assay in order to compare the killing rates of each lethal ovitrap.

Lethal ovitraps

During the intervention, 10 LOs were placed in every house of the block chosen for this treatment. Five LOs were placed inside the house and five were located in open areas (backyard and front yard). The deltamethrin strips in the LOs were changed twice a month due to loss of activity observed in laboratory susceptibility tests of adult *Ae. aegypti* from the study zone (see results). Hay infusion (10% dilution) was added to the LO as an attractant on the first day the traps were set (22). When LOs were serviced and ovistrips were replaced, cups were emptied and refilled with water without cleaning the cup leaving bacterial contents on the wall of the cup. At the time the ovistrips were replaced, the presence of eggs was recorded and the LOs were categorized according to the eggs number (1-10; 11-30; 31-80; >80).

Bti briquettes

In each house, a Bti briquette was placed in the main larval production sites. These were principally ground-level water storage tanks, with the water used for clearing or clothes washing. According to manufacturer's recommendations, a quarter (¼) briquette was placed in each tank, usually of 0.1 to 0.5 m^2 in surface area. A net bag was hung from the tank faucet. Its design allowed maintaining the briquette in the tank with or without water. The briquettes retained their effectiveness even after drying. The briquettes were changed once per month and the condition of the tank and briquette recorded.

Education of the study population before intervention

Two weeks before the intervention, each house in the study neighborhoods was visited by two project members who informed the residents about the dengue problem, the biology of the vector, and the *Ae. aegypti* control methods to be used in the study. A brochure with this information was left in each house. After receiving informed consent to proceed, the project team located mosquito-infested, water-holding containers present in the house, showed them to the residents, and explained the mosquito life cycle. The mosquitoes were removed and the water discarded as part of the clean-up campaign. Families were informed about the re-inspection schedule. The treatment blocks were selected randomly after the visits.

At the end of the 4-month intervention period, a questionnaire was given to an adult family member in the house to evaluate the usefulness of the control methods to the community and to evaluate basic knowledge about dengue virus transmission.

Statistical analysis

Data were entered in an Access database (Microsoft© Office Access, Microsoft Corp., Seattle, WA) and then exported into ArcMap (ver. 8.3, Esreri, Redlands, CA), a geographic information system. For statistical analysis, the house was the sample unit. Data were analyzed using SPSS 7.5 software for windows (SPSS Inc, Chicago 1996) and Stata 6.0 (Stata Corp., 1999). Entomological indices (House and Adult) were expressed as percentages. Comparisons

were tested for significance among interventions (treatments vs. control) and between years (2004 vs. 2005) using Poisson regression. The pupal index was analyzed with a negative binomial regression to compare between the interventions and the first survey. Significance level was set at $p \leq 0.05$ for all statistical analyses.

Results

Efficacy of the lethal ovitraps

Lethal ovitraps produced 97-100% mortality of larvae and adults of the *Ae. aegypti* Rockefeller strain (susceptible strain) for all exposure times (10, 20, 30 days) evaluated under the two environmental conditions (inside the house and open area: shade and sunlight) (table 1). This finding confirmed that water quality did not affect LO insecticide activity. However, when *Ae. aegypti* from the study site (Cali strain) were evaluated, approximately 90% mortality of adults and larvae was achieved at time 0, but after 10 days of exposure to an open environment, mortality significantly decreased (table 2). LOs exposed to both environmental conditions for 10 days prior to efficacy evaluation produced a

mortality rate significantly lower for Cali strain mosquitoes (adults and larvae) compared with the Rockefeller strain (table 2). Mortality rates for the Cali strain of *Ae. aegypti* after 10 days of exposure were: 70.0% for adults and 60.0% for larvae inside a house; and 58.3% for adults and 20.8% for larvae in an open area exposed to sunlight. When mortality was recorded at 45-minute intervals during the assay with the LO exposed 10 days in an open area, significant declines in mortality occurred, as well as an increase in the time required to achieve mortality for the Cali strain compared to the Rockefeller strain (figure 3). Due to the low mortality rates with the Cali strain, the longer exposure times were not evaluated. Biochemical levels for non-specific esterases, mixed function oxidases and insensitive acetylcholinesterase were tested with the *Ae. aegypti* Cali strain, but no significant differences between Rockefeller and Cali strain were noted (data not shown). These results led to a strategy of replacing the LOs deltamethrin strips twice a month during the intervention phase in order to decrease the possibility that the traps would become mosquito production sites (see methodology).

Table 1. Survival rates of larvae or adults of *Aedes aegypti* Rockefeller strain when the LO were exposed at 0, 10, 20 and 30 days under the two environmental conditions (indoors and outdoors).

Time	Adults inside (n=30)	Adults outside (n=30)	P	Larvae inside (n=30)	Larvae-outside (N=30)	P
0	0%	0%	0	0%	0%	0
10	2%	2%	0	0%	0%	0
20	0%	0%	0	10%	3%	0.301
30	0%	3%	0. 313	0%	0%	0

Table 2. Comparison of *Aedes aegypti* adult and larvae survival rates from Cali and Rockefeller exposed to outdoor (sunlight) environmental conditions.

Time (days)	Adults Cali	Adults Rockefeller	P*	Larvae Cali	Larvae Rockefeller	P*
0	9.52% (n=84)	0% (n=10)	0.308	9.3% (n=54)	0% (n=10)	0.316
10	58.33% (n=24)	0% (n=12)	0.001	20.8% (n=24)	0 % (n=12)	0.088
P**	0.001			0.158		

p* indicates the probability between Rockefeller strain and Cali strain to a specific time.
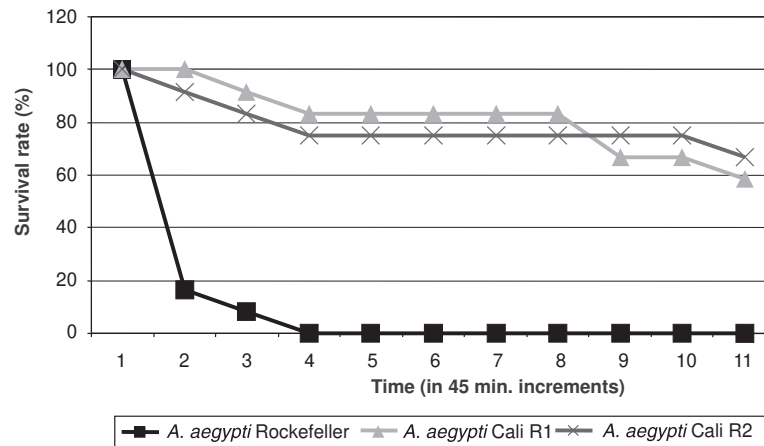p**indicates the probability among the times

Figure 3. Comparison of the adult survival rate, assayed in 45 minute increments, between *Aedes aegypti* Cali strain and Rockefeller strain in LO exposed 10 days to outside environmental conditions. R1 and R2 refer to the number of replicates.

Entomological surveillance pre-intervention

During the six-month survey period (May to October, 2004), 255 houses from *Comuna 16* were sampled. The houses sampled from each district were as follows: 82 houses from "República de Israel" (32.2%), 70 from "Mariano Ramos" (27.5%), 63 from "Unión de Vivienda Popular" (24.7%) and 40 from "Antonio Nariño" (15.7%) (figure 1). The entomological indices varied with precipitation (figure 4). Entomological indices for immature stages of *Ae. aegypti* (house and pupal indices) varied directly with precipitation; in contrast, higher values for the adult index were found in the months with less precipitation (June, July and August) (figure 4). Entomological indices did not differ among the neighborhoods (*p*<0.01).

During the 2004 survey, 69 of 327 containers with water inspected were found positive for immature mosquitos in 49 houses (21.1%). The most common container with water found was low water storage tanks (47%). The percentage of positive containers for larvae and pupae in the houses were principally flower pots or plants in water (33%), low water storage tanks (21.7%), and large miscellaneous containers (20.3%) that were mainly plastic or metallic tanks for water storage. Small volume miscellaneous containers represented only 17.4% of the mosquito-positive

containers and comprised of ceramic plates under the flower pots or plastic containers abandoned in house yards. A few tires (2.9%), bottles (2.9%) and tin cans (1.4%) were also found with water and were positive for *Ae. aegypti* larvae or pupae.

When the containers were analyzed regarding pupal production, the importance of the containers changed. Only 19 of 69 positive containers (27%) contained pupae and were mainly low water storage tanks (36.8%) and large volume miscellaneous containers (31.6%). Flower pots or plants in water comprised only 15.8% (n=3) of the mosquito-positive containers. The pupal index averaged 1.15 pupae per house or 0.2 pupae per person. In the containers, *Ae. aegypti* and *Cx. quinquefasciatus* were found in a ratio of 18.75:1. However, the adult index was 50.2% for *Ae. aegypti* and 41.2% for *Cx. quinquefasciatus*. A mean of 0.33 *Ae. aegypti* adults per person was calculated.

Entomological surveillance during the intervention

During the intervention, reductions in entomo-logical indices achieved in the untreated block was not significantly different (*p*>0.05) from those blocks in which the control measures were implemented (table 3). When the monthly entomological indices obtained from the initial
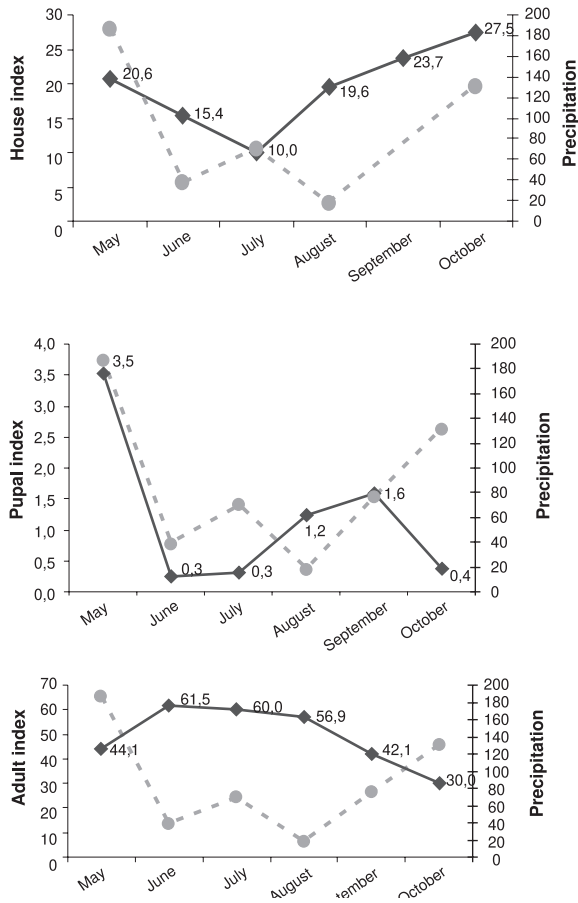
Figure 4. Indices during the survey (2004) in relation to monthly average precipitation. The continuous line corresponds to the indices and point dot line to precipitation. A) House index, b) Pupal index, c) Adult index.

survey in 2004 were compared with the control block in 2005, no significant differences were found. However, when the overall data from the intervened blocks were compared with the 2004 survey, a significant reduction in the number of pupae and adults was detected ($p<0.05$) (table 4). The major effect of all interventions was a reduction in production of pupae. The adult index during the intervention period did not increase later in the season during the low precipitation period, in contrast to the observations in the initial survey (2004). The type of positive containers for immature stages varied. Positive containers consisted principally of plants in water (50%), cans and small volume (<500 ml) miscellaneous

containers (27.5%). Only 2 out of 103 filled low water storage tanks were positive for *Ae. aegypti* immature stages. As expected with this type of positive containers, pupae were not frequently found. Only 18 pupae were collected from 4 houses during the intervention study.

In the treatment blocks with LOs (LO and LO+*Bti*), 961 were placed in 98 houses. From 4,492 service visits of LOs, 45.2% of the strips were positive for *Ae. aegypti* eggs, but only 1.15% of the LOs contained larvae and 0.31% contained pupae; indicating that the immature stages were susceptible to deltamethrin during the fifteen days of the toxic strip evaluation.

The *Bti* briquettes were placed in 76 low water storage tanks and replaced monthly. During the study evaluation (341 visits) only one tank was positive for larvae. In 64% of the visits, the briquette was present in the tank and in 60% of the briquettes were submersed in the water.

Catch basins

Catch basins were evaluated during the pre-intervention and intervention period (table 5).. During the pre-intervention period a higher percentage of the catch basins were positive for pupae and more pupae were collected. In both periods the number of catch basins with *Ae. aegypti* pupae were low. Most of the positive containers with *Ae. aegypti* were found in June.

Knowledge survey

At the end of the study, a brief questionnaire was administered to an adult member of each household that participated in the study. A total of 179 surveys were completed. In terms of dengue knowledge, 81% of the people knew that dengue is transmitted by mosquitoes, 66.5% answered that *Ae. aegypti* is produced in water-holding containers inside the house, and 68% knew that not storing water in their houses was one strategy of mosquito control. With respect to knowledge of interventions, only 29.6% with LOs and 29.5% of the people associated the *Bti* in their houses with control of *Ae. aegypti*. However, 75.3% with LOs and 68.2% of the people with *Bti* perceived reductions in adult mosquito numbers inside their houses; furthermore, 75.3% (LO) and 71.6% (*Bti*)

Table 3. Entomological indices during the pre-intervention (2004) and intervention periods (2005). Statistical analyses compare treatments during 2005; and between 2004 and 2005 control.

Index	Month	2004	2005	2005 Control	OL	OL+	P*	P**
House	May	20.60	10.53	10.00	20.00	15.00	0.608	0.403
	June	15.40	5.00	10.53	0.00	5.88	0.961	0.289
	July	10.00	10.53	5.26	10.00	0.00	0.128	0.953
Pupae per house	May	3.53	0.00	0.10	0.30	0.05	0.679	N/A
	June	0.25	0.00	0.47	0.00	0.00	0.264	N/A
	July	0.30	0.00	0.00	0.00	0.00	N/A	N/A
Adult	May	44.10	42.11	35.00	35.00	50.00	0.560	0.915
	June	61.50	35.00	26.32	25.00	41.18	0.605	0.176
	July	60.00	36.84	26.32	20.00	9.52	0.035	0.256

* p values obtained using Poisson regression, comparing the four treatments in 2005.
** p values obtained using Poisson regression, comparing the 2004 vs 2005 control.

Table 4. Comparison of entomological indices for *Aedes aegypti*: survey (2004) vs intervention (2005).

Index	2004 Index value (n)	2005 Index value (n)	P Value
House	15.1% (19)	8.5% (21)	0.070[a]
Adult	56.3% (71)	34.8% (86)	0.003 [a]
Pupae per house	1.15 (145)	0.073 (18)	0.000 [b]
Total houses sampled	126	247	

[a] Value obtained using Poisson regression.
[b] Value obtained using negative binomial regression

Table 5. Catch basins positive for larvae and pupae during the pre-intervention and intervention period.

Catch basin	total	% Larvae + (n)	Pupae + % Aedes	% Culex	% Both	Pupae Total # (# Ae. aegypti)
Pre-intervention	35	71.4 (25)	2.8 (1)	40.0 (14)	11.4 (4)	1,292 (17)
Intervention	26	57.6 (15)	3.8 (1)	15.3 (4)	11.5 (3)	362 (72)

answered that they would continue using these methods for mosquito elimination.

Discussion

Four interventions in the community using lethal ovitraps, *Bti* briquettes, the combination of both methods and community education were evaluated. The entomological indices obtained during the intervention were not significantly different among the treatments or controls. The entomological indices in the untreated area were not significantly different from the pesticide treatments. This was possibly due to an effect of the education and cleanup campaign at the beginning of the intervention study and the continuous visits by the research team (twice a month). However, a significant reduction in pupae and adult indices was observed between the pre-

intervention survey (2004) and the intervention period (2005). Unfortunately, the decrease in abundance of pupae did not eliminate the presence of adult mosquitoes in the houses to the levels sufficiently low to block transmission. This suggests that the buffer areas surrounding the intervention areas were not large enough to prevent immigration of adult mosquitoes; possibly additional production occurred in cryptic larval habitats not identified in the surveys.

In spite of the small sample size, variation in the types of positive containers, positive houses and adult densities in relation to the intervention and precipitation was observed. Although the sample size is a limitation of the study, it allowed us to identify entomological characteristics from the study sites so that we could directly compare results. During the intervention, the untreated area was affected by some of the same variables affecting mosquito production in the treated blocks (education, cleaning campaign, and periodic visits), which allowed us to decrease the bias and measure the real effect of the pesticide methods.

During the pre-intervention survey, the entomological indices varied over time and were principally affected by precipitation. The indices related to immature stages (house and pupal indices) varied in direct relation with the precipitation. In contrast, the adult index increased during the period of low rainfall. The high abundance of adults suggested that there were other production sites outside the houses during the dry period. This speculation was also supported during the intervention period when pupal production in houses was very low (mean=0.073 pupae per house) but adults persisted in the houses and a reduction of only 30% was observed. The production sites observed for *Ae. aegypti* outside the houses were the catch basins. They have been previously described as permanent breeding sites for *Cx. quinquefasciatus* and *Ae. aegypti* because they permanently maintain water (19,23). In this study, the catch basins evaluated were highly positive for larvae and pupae of *Cx. quinquefasciatus* (71.4%-pre-intervention and 57.6%-intervention) and low percentage of them were positive for *Ae. aegypti* pupae (14.2% and 15.3%, respectively),

principally in June. The low occurrence of *Ae. aegypti* in catch basins during the study, could be explained by the maintenance of the control program with the insecticide Triflumuron which is effective against *Ae. aegypti* but not against *Cx. quinquefasciatus* (24). However, the production of *Ae. aegypti* in catch basins observed in this study does not explain completely the increase of adult frequency in the houses since *Aedes* pupae was found principally in June but not during the entire dry period. Possible explanations are that the number of catch basins examined was low (2 per 10 houses), the presence of other production sites outside the houses (not found in the area covered), or mosquito adult migration from outside buffer zone to treated zone during the intervention period. This last one, suggests the need of a larger buffer zone to prevent immigration and entrance of adults to the study houses. It is well known that *Ae. aegypti* adults mosquitoes can fly long distances (>500 m) (25-27).

During the intervention, the adult index was not significantly different between blocks with LOs and the control block, suggesting that the LO treatment did not reduce the abundance of adults in the houses in comparison with houses with no insecticide treatment but with educational intervention. The presence of adults in treatments areas could be explained as mentioned before due to the small area treated (block and buffer zone) that did not prevent the immigration of adults and/or because of low adult mortality found in the LO bioassays with the local strain of *Ae. aegypti* prior to the intervention study. However, even with the low LO efficacy observed on adults, we observed that the LOs were effective in killing immature stages in the field since 40.8% of the strips were positive for eggs but only 1.4% of LOs contained larvae or pupae which escaped the effect of the insecticide on the strip. This finding suggested that the LO must be exerting a controlling effect on *Aedes* populations, due to the low emergence of larvae observed, but effects on abundance of adults could not be detected in the small area treated. In previous studies using LOs against *Ae. aegypti* in Brazil and Thailand, some adult reduction was found in treated areas, indicating

that LOs do not completely eliminate adults but could be combined with other strategies to achieve effective vector control (28). Our study did not show a clear reduction of adults in LO treated areas in comparison with the control area (education only); however, the low susceptibility of *Ae. aegypti* adults from Cali to the deltamethrin treated strips in LOs suggests that future studies with this methodology should use insecticides with higher efficacy in the laboratory and that LOs should be placed over a larger area to prevent *Ae. aegypti* immigration.

The *Bti* briquette was effective in eliminating *Ae. aegypti* larvae in the low water storage containers since we only found a container with larvae during the intervention period with this control method. Unfortunately, in a high percentage of houses (40%), the briquettes were not used routinely because when they dissolved a visible residue remained in the water, causing people to remove the briquette. This could be the reason for the presence of larvae in the positive container. Since the project team left the briquette and checked them only during the entomological survey, its continued use could not be confirmed. It is possible that in addition to the insecticide effect, its use may also induce a behavioral change in the house inhabitants which clean the water storage containers more often. Although there were no significant differences among treatments, it appears that the combined treatment (LO+*Bti*) achieved a substantial reduction in entomological indices, eliminating positive containers and reducing adult abundance in July (table 3). It is possible that the mosquito reduction was a consequence of the combination of treatments and behavioral changes of the house inhabitants. We speculate that residents learned to better identify the relationship between the presence of larvae and the production of adults and eliminated the potential mosquito breeding sites.

In the knowledge survey, the majority of the people (81%) knew that dengue is transmitted by the mosquito *Ae. aegypti*. However, only 66% of the people interviewed associated the presence of the adult mosquitoes with container habitats of larvae in their houses. In spite of all the activities in the project (education and periodic inspections), only 30% of the inhabitants were able to recognize positive benefits from the treatment methods for vector control. This low percentage perhaps because were not familiar with the methods or mortality of adult mosquitoes was not directly observed. Regardless, they did perceive that adult populations were reduced in their houses.

Results obtained in this study indicate that the area covered in the interventions is not enough to eliminate transmission of dengue viruses in Cali conditions. Additionally, to the small area covered by the treatments, the buffer zone did not work as a barrier for adult mosquitoes. At this level, education and permanent surveillance are as effective in reducing mosquito populations as the insecticidal control methods evaluated. This indicates that future studies should cover larger areas in terms to evaluate the effect of any potential control method to be use by the community.

The low number of pupae found inside the houses during the pre intervention and intervention which was lower than the transmission threshold of 0.5 to 1.5 *Ae. aegypti* per person suggested by Focks and coworkers (28), contrast with the high infestation of adults in houses. These results confirm the presence of breeding sites outside houses in Cali that requires careful investigation. Additionally, the variation of entomological observed during the different precipitation periods in the pre-intervention period, indicates the complexity of the dynamics of the immature stage and adult mosquito populations and the necessity to understand human behavior, mosquito population dynamics and the production potential of sites inhabited by immature *Ae. aegypti* inside and outside houses.

Acknowledgment

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Anexos

Cuestionario sobre aceptación del proyecto sobre comparación de dos métodos de control en la ciudad de Cali

Fecha: __ __ __ / __ __ __ __
        DÍA   MES   AÑO   Barrio:_____

Dirección:_____Código:_____Piso:_____

Encuestador:_____

Nombre de la persona encuestada:_____

Relación con el jefe de hogar: _____ Edad: _____ (años)

¿Sabe leer?   1. Sí   2. No

1.   ¿Sabe usted cómo se transmite el dengue?

    ☐ 1. Picadura de mosquito   ☐ 3. No sabe.
    ☐ 2. Otro (¿Cual?)_____   ☐ 4. No responde.

    Si la respuesta es correcta ir a 2, si es incorrecta ir a 3.

2.   ¿Sabe dónde se cría el mosquito *Aedes Aegypti*, vector del dengue? (<u>opción múltiple</u>)

    ☐ En recipientes con agua en las casas   ☐ Otro (¿Cuál?)_____
    ☐ En los sumideros   ☐ No sabe.
    ☐ En los caños   ☐ No responde.

3.   ¿Qué podemos hacer para evitar el dengue? (<u>opción múltiple</u>)

    ☐ Matar los mosquitos con insecticida   ☐ Otro (¿Cuál?)_____
    ☐ Destruir los criaderos   ☐ No sabe.
    ☐ No almacenar recipientes en las casas ☐ No responde.
    ☐ Tener seco el lavadero

4.   ¿De dónde proviene el agua que utiliza normalmente?

    ☐ 1. Acueducto   ☐ 2. Lluvia

5.   ¿Usted almacena agua en su casa?

    ☐ 1. Sí   ☐ 2. No

    Si la respuesta es "sí" ir a 6, si es "no", ir a 9.

6.   ¿Durante cuánto tiempo la almacena?

    ☐ 1. 1 a 3 días   ☐ 4. No sabe.
    ☐ 2. 4 a 7 días   ☐ 5. No responde.
    ☐ 3. Más de una semana

7.   ¿En dónde la almacena? (<u>opción múltiple</u>)

    ☐ Tanque del lavadero
    ☐ Tanque plástico, metálico o cerámico
    ☐ Otro (¿Cuál?)_____

8.   ¿Mantiene tapados estos tanques?

    ☐ 1. Sí   ☐ 2. No

EN LAS CASAS CON OVITRAMPAS

9.   ¿Sabe usted para qué son los vasos negros que se pusieron en su casa? (opción múltiple)

☐   Para matar mosquitos   ☐   No sabe.
☐   Para recolectar huevos de mosquitos   ☐   No responde.
☐   Otro (¿Cuál?)_____

Si no sabe o no responde, explicar el objetivo de los vasos.

10.   ¿Le parece que con este tratamiento se redujo el número de mosquitos durante el día?

☐   1. Sí   ☐   3. No sabe.
☐   2. No   ☐   4. No responde.

11.   ¿Usaría usted estos vasos en su casa para controlar los mosquitos?

☐   1. Sí. ¿Por qué?_____

_____

☐   2. No. ¿Por qué?_____

_____

12.   ¿Los utilizaría si usted fuera quien cambiara la tira de insecticidas periódicamente?

☐   1. Sí. ¿Por qué?_____

_____

☐   2. No. ¿Por qué?_____

_____

Comentarios adicionales sobre las OL   _____

_____

_____

EN LAS CASAS CON BTI

13.   ¿Sabe usted para qué son las pastillas que se pusieron en su lavadero?

☐   1. Para matar las larvas del mosquito
☐   2. Otro (¿Cuál?)_____
☐   3. No sabe.
☐   4. No responde.

Si no sabe o no responde, explicar el objetivo de la pastilla.

14.   ¿Le parece que con este tratamiento se redujo el número de mosquitos durante el día?

☐   1. Sí   ☐   3. No sabe.
☐   2. No   ☐   4. No responde.

15.   ¿Usaría usted la pastilla permanentemente en su casa para controlar los mosquitos?

☐   1. Sí. ¿Por qué?_____

_____

☐   2. No. ¿Por qué?_____

_____

16.   ¿Los usaría si usted fuera quien cambiara las pastillas periódicamente?

☐   1. Sí. ¿Por qué?_____

_____

☐   2. No. ¿Por qué?_____

_____

Comentarios adicionales sobre el Bti

_____

_____

_____

FORMULARIO CASAS DEL MUESTREO

Importante: Llenar TODOS los campos del formulario   **CÓDIGO:** _____

Fecha: _____ _____ _____   **Barrio:** _____   **Funcionario:** _____
        Día   Mes   Año

Dirección: _____ **Piso:** _____

Datos de la vivienda	Adultos #	Niños #	Cuartos #	Patios #	Terraza: ☐ 1. Sí ☐ 2. No	Uso de insecticidas: ☐ 1. Diariamente ☐ 2. Semanalmente ☐ 3. Quincenalmente	☐ 4. Mensualmente ☐ 5. Mas de 1 mes ☐ 6. Nunca	Marca del insecticida:

Datos criaderos

#	Tipo*	¿Cuál? (Ilenar únicamente para "Diversos" 9-10)	Volumen) (ml	Larvas 1. Positivo 2. Negativo	Pupas #	*Culex* spp Machos Hembras	*Aedes aegypti* Machos Hembras	*Tipos de criaderos:
1.								Tanques altos
2.								Tanques bajos
3.								Llantas
4.								Tina
5.								Florero
6.								Mata en agua
7.								Tarros, latas
8.								Criaderos naturales
9.								Diversos ≤ 500 ml (¿Cuál?)
10.								Diversos >500ml (¿Cuál?)

Datos adultos	*Culex* spp	Machos	Hembras	*Aedes aegypti*	Machos	Hembras	*Aedes aegypti* Hembras		
							Nula	Para	Con sangre / Sin sangre

Ovitrampa letal

#	Ubicación 1. Adentro 2. Afuera	Huevos**	Larvas** Estadio 1 #	Estadio 2 #	Estadio 3 #	Estadio 4 #	Pupas #	Observaciones
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

	Bti: 1. Presente 2. Ausente	Ubicación: 1. Dentro del agua 2. Fuera del agua	Larvas** Estadio 1 #	Estadio 2 #	Estadio 3 #	Estadio 4 #	Pupas #	**Productividad de larvas y huevos:
→ Bti Tanque 1								1. Cero (0)
Tanque 2								2. de 1-10
								3. de 11-30
								4. de 31-80
								5. > 80