Towards a *Casa Segura*: A Consumer Product Study of the Effect of Insecticide-Treated Curtains on *Aedes aegypti* and Dengue Virus Infections in the Home

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Abstract. The home, or domicile, is the principal environment for transmission of dengue virus (DENV) between humans and mosquito vectors. Community-wide distribution of insecticide-treated curtains (ITCs), mimicking vector control program-driven interventions, has shown promise to reduce DENV infections. We conducted a *Casa Segura* consumer product intervention study in Mérida, Mexico to determine the potential to reduce intradomicillary DENV transmission through ITC use in individual homes. Dengue virus infections in mosquitoes and in humans were reduced in homes with ITCs in one of two study subareas. Overall, ITCs reduced intradomicillary DENV transmission; ITC homes were significantly less likely to experience multiple DENV infections in humans than NTC homes. Dengue virus-infected *Aedes aegypti* females were reduced within the ITC homes where curtain use was highest. Some homes yielded up to nine infected *Ae. aegypti* females. This study provides insights regarding best practices for *Casa Segura* interventions to protect homes from intradomicillary DENV transmission.

INTRODUCTION

Epidemic dengue has emerged in the Americas and has had devastating public health consequences. Dengue hyperendemicity, with co-circulation of all four dengue virus (DENV) serotypes in many areas, has resulted in increases in the number of dengue cases with severe manifestations.^{1–3} Because there are no vaccines or therapeutics yet available for use against DENV, targeting the vector(s) is the only approach presently available for dengue control. In most settings, the primarily day-biting mosquito *Aedes aegypti* is the principal vector of DENV.^{4,5} Many countries, including Mexico, rely on environmental sanitation and source reduction to remove larval development sites and space spraying of insecticides to eliminate adults in and around homes.⁴ Although beneficial, these measures have not proven to be sufficient or sustainable to stem the rising tide of dengue in the Americas.^{3,6} New tools and approaches therefore are needed to help control dengue.^{7,8}

Our previous studies in Mérida⁹ incriminated the home as an important environment for DENV transmission between humans and *Ae. aegypti*. Our emerging model of DENV transmission distinguishes between intradomicillary virus transmission and amplification, which occurs in the indoor home environment, and extradomicillary virus transmission, which may occur either in other indoor environments or outdoors.¹⁰ One approach for dengue control is to use insecticide-treated materials deployed as curtains (insecticide-treated curtains [ITCs]) to preclude the endophilic and endophagic *Ae. aegypti* from the indoor home environment and thus to interrupt intradomicillary DENV transmission.^{11–21} Because of the endophilic nature of *Ae. aegypti*, tools or approaches to exclude it from the epidemiologically most significant points of contact with humans, the home and

other frequently used indoor environments, such as schools or workplaces,^{9,22} would be an important addition to the armamentarium for dengue control. This type of intervention theoretically not only prevents DENV-infected female mosquitoes from entering homes and other indoor environments, such as schools or workplaces, but also prevents mosquitoes from feeding indoors on viremic humans, including while they are recuperating in the home, and then transmitting the virus to other humans frequenting the domicile. The intervention thus could interrupt virus transmission at two points in human–mosquito DENV transmission. We have designated the use of ITCs to protect homes as the *Casa Segura* (safe house) approach.¹⁰

The previously mentioned studies on the use of ITCs to protect the home from *Ae. aegypti* and intradomicillary DENV transmission were most commonly conducted as community-based interventions often based on the cluster-randomized trial design.²³ This mimics a public health driven vector control program intervention with most of the homes in an intervention cluster receiving the ITCs. The success of these previous studies may, in part, be related to a community control effect resulting from ITCs being placed in most homes and protecting nearby homes without ITCs. In our study design, we focused on individual homes, rather than clusters of homes, to investigate the potential for a consumer product *Casa Segura*, in which individual homeowners use ITCs to reduce the risk of intradomicillary DENV transmission by *Ae. aegypti* in their respective homes. Intervention homes with ITCs and control homes with non-treated window curtains (NTCs) were purposefully located far enough from each other to reduce any community effect associated with nearby homes having ITCs. Study homes were monitored for virologic outcomes, including DENV infections in study participants living in the homes and in *Ae. aegypti* females collected from within study homes, as well as other pertinent epidemiologic and entomologic outcomes. Our studies showed the importance of targeting the home to prevent intradomicillary DENV transmission and provided information regarding best practices for implementation of *Casa Segura* interventions.

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MATERIALS AND METHODS

Study approach and design. This study was designed to determine the potential for a consumer product *Casa Segura* approach to protect individual homes from dengue and *Aedes aegypti*. In brief, candidate cement style homes in *fraccionamiento* neighborhoods of Mérida, Mexico were identified and randomly selected; families were approached to participate, and if agreeable informed consent was obtained from participants. Enrolled homes were outfitted with ITCs or NTCs, and DENV infections in humans and *Ae. aegypti*, as well as mosquito abundance, were monitored in the intervention and control homes during a year-long study. Mosquitoes were monitored for susceptibility to the ITCs and for presence of a knock-down resistance-conferring allele; the type and frequency of insecticide use in homes were also determined.

A power analysis showed that a two-arm study design comprising 400 homes (200 intervention homes fitted with ITCs and 200 control homes fitted with identical but NTCs, and with approximately five persons per home) and assuming a reasonable effect size of 5% (reduction in infections from 10% to 5%) would have at least 95% power to detect a statistically significant reduction in infection rates in ITC versus NTC homes. We also assumed that there would be no or minimal inter-home correlation because the study homes were located minimally 80–100 meters apart, which exceeds the typical flight range of *Ae. aegypti* in urban settings,²⁴ to minimize spillover or community effects from ITC treatment to NTC control homes. Intervention and control homes were then monitored for a year, and human DENV infections and *Ae. aegypti* abundance and DENV infection rates were compared between ITC and NTC homes.

The study was approved by the Institutional Review Board of Colorado State University and the Bioethics Committee of Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán.

Study environment and home inclusion/exclusion criteria. The study was conducted in the subtropical city of Mérida (population ≈800,000), which is located in the Yucatán peninsula of Mexico. Mérida is hyper-endemic for dengue, with co-circulation of multiple DENV serotypes, and *Ae. aegypti* is the primary mosquito vector.^{9,22,25,26} Study areas were identified for similarity in potential study homes, e.g., cement construction, size, and design, typically found in *fraccionamiento* neighborhoods, which are comprised of high-density and low-income housing. Homes with more than one story, more than 10 windows, less than four inhabitants, or no children < 15 years of age were excluded from the study. Matched intervention and control homes were typically within a single block or nearby in the respective neighborhood. Initially, homes were to be in neighborhoods in southern Mérida (San Carlos, Serapio Rendon, Brisas del Sur, Villa Magna, Zazil-Ha, Nicolás del Sur, and Hacienda). However, because of the exclusion criteria, it was necessary to recruit additional homes in the Vergeles neighborhood of eastern Mérida to achieve the overall number of homes determined via the power analysis to be required for the study. All study homes were equipped with electricity and running water. The two major study areas were designated subareas South and East.

Selection of homes and enrollment of participants. Google Earth images of the neighborhoods were used to identify blocks with potential intervention and control homes. Candi-

date home owners were approached to enroll in the study by trained nurses who informed the family members of the intent and scope of the study (including the need to provide blood samples) using a prepared script and an informed consent form approved by the Ethics Committee of Universidad Autónoma de Yucatán and the Colorado State University Institutional Review Board. If the family agreed to participate, all family members > 1 year of age provided consent and were enrolled in the study; parents signed the consent form for minors after they agreed to participate. The first home enrolled on a block received ITCs. A second home on the same block or nearby in the neighborhood, separated by > 80 meters from the ITC home, was then randomly identified, and the family was approached to participate in the study. If they agreed, informed consent was obtained from the participants, and that home received NTCs. This study design was chosen in part because the Colorado State University Institutional Review Board required, as part of the consent process, that the families be told whether the curtains to be installed contained the insecticide.

A total of 411 homes were included in the study; 206 of these received ITCs and 205 received NTCs. A total of 1,778 occupants consented and were enrolled into the study and provided a blood sample for testing of exposure to DENV on at least one occasion.

Curtain installation. After enrollment of families into the study, homes were equipped with ITCs (206 intervention homes) or NTCs (205 control homes) as noted above. Curtains were installed, during May–September 2009, in all windows of the study homes, and the participants were informed if their home received ITCs or NTCs. No effort was made to protect doorways in this study. The insecticide-treated and non-treated materials used to produce the window curtains for the study were provided through a joint effort by Acytex Internacional (México City, Mexico) and Bayer de México (México City, Mexico). The active ingredient for the treated material, which had a mesh size of 2×2 mm, was deltamethrin at a targeted concentration of 80 mg/m². The killing efficacy of the ITCs for *Ae. aegypti* is described in the section Deltamethrin concentration in and killing efficacy of ITCs.

Monitoring study participants for DENV infection. Study participants were monitored for DENV infection through serosurveys. We also captured dengue disease cases among the study population based on laboratory confirmation by the Laboratorio Estatal de Referencia Epidemiológica (Epidemiological Reference Laboratory of Yucatán State).

Serosurvey schedule. Serosurveys were conducted A) at the time the curtains were installed (baseline sample; May–September 2009); B) after the peak of the 2009 DENV transmission season (first post-curtain installation sample; November 2009–January 2010); and C) in the summer of the following year (second post-curtain installation sample; July–August 2010). This schedule corresponded to the two post-curtain installation study periods of the project. Study period 1 (SP1) covered the time of historically most intense DENV transmission in Mérida from late summer through the end of the year (September–December 2009). Study period 2 (SP2) was from the beginning to the early summer of the following year (January–July 2010), which corresponds to a time period with historically lower DENV transmission intensity.

Blood/serum samples. Blood samples were obtained by trained nurses. For children ≤ 5 years of age, blood samples

were obtained through finger prick by using the Accu-Chek system (Roche Diagnostics, Indianapolis, IN) and heparinized micro-hematocrit tubes. For those > 5 years of age, samples were obtained by either finger prick, as described above, or by venipuncture using a sterile needle and a 5-mL Vacutainer tube. Plasma was obtained by centrifugation and stored at -70°C until tested for exposure to DENV.

Dengue disease. Homes were visited monthly by nurses who asked about the health status of the participants. If participants with a fever or who had a fever since the previous visit were identified, they were encouraged to visit the local health clinic for laboratory diagnosis. Records of dengue disease among these study participants were based on laboratory confirmation by the Epidemiological Reference Laboratory of Yucatán State.

Diagnostic tests for DENV infections. Study participants were monitored for dengue infection and seroconversion by using a combination of standard serologic and virologic tests. An IgM-capture enzyme-linked immunosorbent assay was used to detect recent DENV infections, including all available baseline, serosurvey 1, and serosurvey 2 specimens.²⁷ Acute-phase samples were processed for DENV isolation by infection of cultured C6/36 cells, and isolated DENVs were identified by using immunofluorescence.²⁸ DENV RNA was also amplified from acute-phase specimens and serotypes were identified by using reverse transcription-polymerase chain reaction (RT-PCR).^{29,30} A $\geq 90\%$ plaque reduction neutralization test (PRNT) was used to detect seroconversions using baseline, serosurvey 1, and serosurvey 2 specimens for persons who provided specimens on at least two of these occasions.³¹ A participant was considered to have been infected with DENV during the course of the study if he or she had positive results in one or more of these laboratory tests. Finally, because of our specific interest in infection in young children, participating children also were assayed for DENV infection before the study by detection of DENV IgG by using a commercially available kit (Dengue IgG-indirect Kit; Panbio Diagnostics, Brisbane, Queensland, Australia).

Monitoring homes for mosquitoes and DENV infection in *Ae. aegypti* females. The mosquito-based outcome measures included indoor mosquito abundance, as well as virologic outcomes in the form of infection prevalence of *Ae. aegypti* females with DENV and indoor abundance of DENV-infected *Ae. aegypti* females.

Collection of adult mosquitoes. Mosquitoes were collected using CDC style backpack aspirators.³² Collections were conducted from 8:00 AM through 3:00 PM and included all rooms in the homes and the outdoor patio. This collection included aspiration from furniture and behind hanging clothes and curtains. The length of time spent collecting per home typically was approximately 20 minutes. Collections were conducted at the time the curtains were installed (baseline sample: May through early September 2009) and then approximately monthly after curtain installation during September 2009–July 2010. Mosquitoes were identified to species by using stereo microscopes and published identification keys.^{33,34} A cold chain was maintained from the collection and transport to the laboratory through the species identification and DENV detection processes.

Dengue virus detection in *Ae. aegypti* females. Mosquitoes were assayed by RT-PCR for the presence of DENV RNA as described^{9,22} with the following modification. We first triturated females individually and then pooled the resulting samples in pools containing up to 10 females for RNA extraction and DENV detection. For pools positive for DENV RNA, we

then re-processed individual females for DENV detection. Dengue virus was amplified and visualized as described above for human serum samples. This procedure enabled us to determine DENV infection prevalence based on positive individual mosquitoes, which is preferable to estimates based on positive pools. The testing included the entire mosquito specimen, and the results therefore should be interpreted as a positive mosquito being infected with DENV but having an unknown status with regards to infectiousness to humans. Finally, mosquitoes that were positive by RT-PCR for DENV RNA were processed for virus isolation in C6/36 cells as described above for virus isolation from human blood samples.

Additional investigations. We also determined some additional study outcomes relating to 1) insecticide resistance of *Ae. aegypti*, 2) changes to the concentration of active ingredient (deltamethrin) and killing efficacy of the ITCs for *Ae. aegypti* over time, and 3) behavior of the study participants with regards to personal protection measures against mosquitoes.

Presence of knock-down resistance–conferring mutations in *Ae. aegypti*. A previous study reported a rapid increase in Mexico, including Mérida, in the past decade of a mutation (the Ile 1,016 allele) in *Ae. aegypti* that confers knock-down resistance to synthetic pyrethroids.³⁵ The presence of this knock-down resistance–conferring allele could potentially increase the likelihood of *Ae. aegypti* surviving contact with the deltamethrin-treated window curtains and penetrating the ITC barrier into the home. To assess this potential confounder for the study outcome, we determined the frequencies of the Ile 1,016 allele in collections of *Ae. aegypti* from ITC versus NTC homes. The method for detection of the Ile 1,016 allele in *Ae. aegypti* followed that described by Ponce Garcia and others.³⁵

Deltamethrin concentration in and killing efficacy of ITCs. The ITCs were placed in south-facing and north-facing windows of study homes during May–June 2009, and ITC samples hung with the ITCs were assayed for deltamethrin concentration and killing efficacy for *Ae. aegypti* during May–June 2009 and in June 2010 at the end of the study. Deltamethrin concentrations were determined at Bayer Environmental Science/BioGenius (Leverkusen, Germany). In brief, for three homes with linked data for deltamethrin concentration and killing efficacy of ITC samples for the two time points, deltamethrin concentration of samples collected during May–June 2009 averaged 2.56 g/kg and decreased after exposure in study home windows to an average of 2.07 g/kg in samples collected during June 2010. Killing efficacy of the ITC samples was determined by using World Health Organization cone bioassays for a local Mérida strain of *Ae. aegypti* and the New Orleans strain of *Ae. aegypti*, which is susceptible to pyrethroid insecticides. At the time treated curtains were first placed in the homes during May–June 2009, their killing efficacy in the standard World Health Organization cone bioassay was 77.3% for the local Mérida strain of *Ae. aegypti* and 100% for the New Orleans reference strain. For the ITC samples collected during June 2010, the killing efficacy was 63.3% for the local Mérida strain of *Ae. aegypti*, but unfortunately the New Orleans strain had been lost in the insectary at Mérida and thus was not included as a reference strain for the bioassay. Clearly, the ITC samples remained lethal to the colonized Mérida strain of *Ae. aegypti* after a year in the study homes. However, it should be noted that the insecticide susceptibility of the colonized local mosquito strain may have increased over time in the absence of insecticide pressure.

Curtain use and personal protective measures. We realized in the fall of 2009 that curtains were commonly not used as intended in the study homes, most notably being removed or tied up and thus leaving the window unprotected or poorly protected against mosquito intrusion. During 2010, monthly entomology team visits therefore incorporated a basic assessment of curtain use. For each entomology team visit when data were collected for curtain use, a value was calculated for a Curtain Use Index (CUI) of that home during that particular visit: $\text{CUI} = \text{no. windows with optimally used curtains} / \text{total no. windows}$. Optimal use of a study curtain was defined as the curtain being present and extended (covering the window) and not covered by another non-treated curtain (typically a privacy curtain), which would aid mosquitoes in entering or exiting homes. Based on the data available for the CUI, we then classified houses by quartiles of their mean CUI as limited (0–0.100), low (0.101–0.230), medium (0.231–0.339), or high (0.340–1).

Personal protective measures used indoors against mosquitoes were examined through a brief questionnaire for most households during February–March 2010 that posed the following questions: 1) Are you taking action to kill mosquitoes inside the house (Yes or No)?; 2) Which methods are you using to kill mosquitoes inside the house (Spray cans, Mosquito coils, Electric plug-ins, Other methods)?; and 3) How often do you use these things in the house to kill mosquitoes (Every day, Every week, Sometimes, Never, Not sure)?.

Statistical analyses. Mosquito-based outcomes. Numbers of female *Ae. aegypti* and *Culex quinquefasciatus* mosquitoes, and DENV-infected *Ae. aegypti* females inside the study homes were statistically evaluated via repeated-measures analysis of variance (ANOVA) with fixed main effects for study subarea (South or East), treatment (ITCs or NTCs), and month of collection. To better satisfy the usual assumptions of normality and homoscedasticity for the ANOVA, the analyses were based on log-transformed data – specifically, because of the presence of zero counts, $\log_{10}(\text{count} + 1)$. The general linear model (GLM) for each log-count variable in this study can be expressed as (1) $Y = \mu + T + A + \text{Tx}A + \varepsilon + M + \text{Tx}M + \text{Ax}M + \text{Tx}A\text{x}M + \delta$, where Y denotes the log-transformed mosquito count variable, μ denotes the overall mean, and T and A denote the fixed effects for treatment and study subarea, respectively, M denotes the fixed effect of time (equivalently, month of collection), and ε and δ denote random variability among homes and within homes over time, respectively. Interactions are shown as products of model terms.

Curtain use. In addition, to account for possible variation in the use of curtains during the study, homes were categorized according to the mean of the values for the CUI obtained from monthly home visits during February–July 2010; the same log-transformed mosquito counts were statistically evaluated via repeated-measures ANOVA with the addition of a fixed main effect for curtain use (as quartiles of the mean CUI for each home). Thus, the GLM for each log-count variable in this secondary analysis can be expressed as (2) $Y = \mu + T + A + C + \text{Tx}A + \text{Tx}C + \text{Ax}C + \text{Tx}A\text{x}C + \varepsilon + M + \text{Tx}M + \text{Ax}M + \text{Cx}M + \text{Tx}A\text{x}M + \text{Tx}C\text{x}M + \text{Ax}C\text{x}M + \text{Tx}A\text{x}C\text{x}M + \delta$, where Y , μ , T , A , V , ε , and δ are the same as defined in (1) above and C denote the fixed effect for curtain use. As above, interactions are shown as products of model terms. Data on each log-count response variable were analyzed according to the above two GLMs via application of the MIXED procedure in SAS software (SAS Institute, Cary, NC) for repeated-measures

ANOVA with the autoregressive order 1, AR(1), and structure of the covariance matrix for the repeated-measures. Least-squares means along with their standard errors, in particular, for the two treatment groups both overall and by subarea, CUI quartile, and month of collection, were obtained via the respective final ANOVA models. Pairwise comparisons of treatment group means were based on Fisher's least-significant-difference method and were carried out only when the statistical significance of the ANOVA test for either the main effect for treatment or an interaction involving treatment was ≥ 0.10 .

In addition, the prevalence of DENV infection in *Ae. aegypti* females collected within ITC or NTC homes in the South or East study subareas was compared between ITC and NTC homes based on the standard Normal Z-test for the difference in two proportions, albeit, ignoring the repeated sampling of the study houses over time.

Dengue virus infection in humans. The following data on binary response variables (Yes/No) taken as evidence of human DENV infection were obtained from as many members of each study household as possible. To determine baseline DENV infection status, serum samples taken before curtain installation were assayed for DENV-specific IgM, IgG or PRNT; results were denoted as DENV + BG for purposes of statistical analyses. Evidence (IgM, PRNT, virus isolation, or RT-PCR amplification positive) of DENV infection for the post-curtain installation Study Period 1 (SP1) or Study Period 2 (SP2) were denoted as DENV+ SP1 and DENV+ SP2, respectively, for purposes of statistical analyses. Evidence of DENV infection for the entire post-curtain installation part of the study (SP1 + SP2) was denoted as DENV + SP1 and 2 for purposes of statistical analyses.

Data on each of these binary variables was statistically evaluated via multiple logistic regression (MLR) analysis with fixed main effects for subarea of study and treatment according to the GLM (3) $Y = \mu + T + A + \text{Tx}A + \varepsilon + \delta$, where Y represent the logit-transform, μ , T , and A are the same as defined in (1) above, and ε and δ denote random variability among homes and among persons within houses, respectively. The interaction of treatment and study area is denoted as $\text{Tx}A$.

In addition, as in the statistical analysis of the mosquito-based data, data on each of the above binary variables indicative of human infection with DENV were statistically evaluated via MLR analysis with the addition of a fixed main effect for CUI (as quartiles of the mean CUI for each house). Thus, the GLM for each binary variable in this secondary analysis can be expressed as (4) $Y = \mu + T + A + C + \text{Tx}A + \text{Tx}C + \text{Ax}C + \text{Tx}A\text{x}C + \varepsilon + \delta$, where Y , μ , T , A , ε , and δ are the same as defined in (3) above and C denote the fixed effect for CUI. As above, interactions are shown as products of model terms.

Data on each binary response variable were analyzed according to the above two GLMs via application of the GLIMMIX procedure in SAS software for mixed-model MLR analysis. The Kenward-Rogers option was used to accommodate the most likely non-significant interactions between fixed and random effects. Pairwise comparisons of treatment group means were based on Fisher's least-significant-difference method and were carried-out only when the statistical significance of the ANOVA test for either the main effect for treatment or an interaction involving treatment was ≥ 0.10 .

Finally, the prevalence of dengue virus infection in residents of ITC or NTC homes in the South or East study subareas after curtain installation was compared between ITC

and NTC homes based on the standard Normal Z-test for the difference in two proportions, albeit, ignoring the clustering of study subjects within study houses.

RESULTS

South and east study subareas: differences in dengue virus transmission intensity. Study homes were selected in subareas South ($n = 306$ homes) and East ($n = 105$ homes), which historically have experienced DENV activity. To determine if DENV transmission intensity was similar in these two subareas during the study period, and data thus could be combined in the analyses, DENV infections in mosquitoes or humans were compared between the subareas. *Ae. aegypti* females collected from homes in the East were significantly ($P = 0.001$) more likely to be infected with DENV than those collected from homes in the South (Table 1). Similar results were found for DENV infection in humans: study participants in the East were significantly more likely to have been exposed to DENV compared with those residing in the South (Table 2). Because of these differences in DENV transmission intensity, data for virologic outcomes (DENV infection in humans or mosquitoes) from the two study subareas were analyzed with any potential difference between subareas accounted for as a model effect via stratification.

Mosquito collections and curtain use. Numbers of collected mosquitoes were recorded from inside 394 study homes, each sampled before curtain installation and then up to 11 times (approximately every month) post-curtain installation. In all, count data for male and female *Ae. aegypti* and *Cx. quinquefasciatus*, and DENV-infected *Ae. aegypti* females, were obtained from 3,499 household sampling occasions. Our collection effort produced totals of 17,976 *Ae. aegypti* (10,254 females and 7,722 males) and 20,010 *Cx. quinquefasciatus* (12,595 females and 7,415 males).

Data on use of the study curtains, obtained for 347 study homes during February–July 2010, were used to develop a CUI corresponding to each of the mosquito collection occasions in a given home during this time period. The mean CUI for each of these 347 study homes was used to determine CUI quartiles to be included as a main effect in the ANOVA of log-transformed counts of *Ae. aegypti* females, *Cx. quinquefasciatus* females, or DENV-infected *Ae. aegypti* females, for which there were 3,372 sampling occasions of mosquitoes.

Virologic outcomes for mosquitoes. Key virologic outcomes for mosquitoes, including overall DENV infection in *Ae. aegypti*

TABLE 1

Prevalence of dengue virus infection in *Aedes aegypti* females collected within homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) in south or east study subareas in Mérida, Mexico

Subarea	Prevalence of dengue virus infection in <i>Ae. aegypti</i> females		
	NTC homes	ITC homes	All homes
Mosquitoes collected pre-curtain installation			
South	0.7% (3/449)	0.0% (0/210)	0.5% (3/659)
East	0.0% (0/598)	0.0% (0/186)	0.0% (0/784)
Total	0.3% (3/1,047)	0.0% (0/396)	0.2% (3/1,443)
Mosquitoes collected post-curtain installation			
South	0.3% (7/2,531)	0.5% (17/3,627)	0.4% (24/6,158)
East	2.2%* (40/1,843)	0.3%* (2/713)	1.6% (42/2,556)
Total	1.1% (47/4,374)	0.4% (19/4,340)	0.8% (66/8,714)

*Significant difference between NTC and ITC homes ($P = 0.001$).

TABLE 2

Prevalence of dengue virus infection in humans residing in homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) in south or east study subareas in Mérida, Mexico

Subarea	Prevalence of dengue virus infection in humans*		
	NTC homes	ITC homes	All homes
Pre-curtain installation: recent infections (IgM)			
South	0.2% (1/465)	0.3% (2/723)	0.3% (3/1,188)
East	0.9% (3/325)	0.0% (0/71)	0.8% (3/396)
Total	0.5% (4/790)	0.3% (2/794)	0.4% (6/1,584)
Pre-curtain installation: all previous infections (multiple detection methods)			
South	17.3% (81/467)	23.6% (172/729)	21.2% (253/1,196)
East	36.2% (118/326)	35.2% (25/71)	36.0% (143/397)
Total	25.1% (199/793)	24.6% (197/800)	24.9% (396/1,593)
Post-curtain installation: recent infections (multiple detection methods)			
South	7.3% (30/413)	7.1% (45/636)	7.1% (75/1,049)
East	24.2% (76/314)	18.6% (16/86)	23.0% (92/400)
Total	14.6% (106/727)	8.4% (61/722)	11.5% (167/1,449)

*No statistically significant difference between NTC and ITC homes ($P > 0.05$).

females, determination of DENV serotypes and clustering of DENV-infected *Ae. aegypti* females, are described below.

Overall DENV infection in *Ae. aegypti* females. The prevalence of DENV infection in *Ae. aegypti* females collected indoors was similar among ITC and NTC homes in both study subareas before curtains were installed (Table 1). In the East, *Ae. aegypti* females collected indoors post-curtain installation from NTC homes were significantly ($P = 0.001$) more likely to be infected with DENV compared with those collected from ITC homes (Table 1). In the South, DENV infection prevalence was similar for mosquitoes from NTC and ITC homes with a slightly higher infection prevalence in ITC homes (Table 1). Similarly, there was a post-curtain installation trend towards a higher percentage of NTC homes versus ITC homes with DENV-infected *Ae. aegypti* females collected indoors in the East (18.1% of 83 NTC homes and 10.0% of 20 ITC homes) but not in the South (5.2% of 114 NTC homes and 5.2% of 172 ITC homes).

Multiple DENV-infected *Ae. aegypti* females were detected within nine study homes post-curtain installation. This included four ITC homes, which averaged 2.5 infected mosquitoes per home (range = 2–3), and five NTC homes averaging 6.2 infected mosquitoes per home (range = 2–9). Dengue virus-infected *Ae. aegypti* females ($n = 66$) were collected most commonly from bedrooms (47.0%) and living/dining rooms (36.4%). Bedrooms in ITC homes tended to account for a greater proportion of collected DENV-infected *Ae. aegypti* females (57.9%, 11 of 19) compared with NTC homes (42.6%, 20 of 47), although the difference was not statistically significant ($P = 0.258$).

DENV infection in *Ae. aegypti* females by serotype. Including all DENV-infected *Ae. aegypti* females collected (pre- or post-curtain installation, and indoors or on the patio), we recorded all 4 DENV serotypes in the East but only DENV-1 and DENV-2 in the South (Table 3). The presence of DENV-3 and DENV-4 in mosquitoes in the East could, in part, have conditioned the increased DENV transmission intensity in this subarea. Importantly, the detection of all four DENV serotypes in one transmission season demonstrates dengue hyperendemicity in the Yucatán.

Repeated measures analysis for clustering of DENV-infected *Ae. aegypti* females in homes. The log-transformed count of

TABLE 3

Dengue virus (DENV) infections of *Aedes aegypti* females by serotype in south or east study subareas in Mérida, Mexico

Subarea	Total no. DENV-infected <i>Ae. aegypti</i> females collected	Percentage (no.) infected by DENV serotype			
		DENV-1	DENV-2	DENV-3	DENV-4
East	45	84.4 (38)	6.7 (3)	2.2 (1)	6.7 (3)
South	28	60.7 (17)	39.3 (11)	0 (0)	0 (0)
Total	73	75.3 (55)	19.2 (14)	1.4 (1)	4.1 (3)

DENV-infected *Ae. aegypti* females collected in the study homes differed significantly between the two study subareas ($P = 0.039$) and among the post-curtain installation collection months ($P = 0.002$), but not between ITC and NTC homes ($P = 0.107$). Because of a statistically significant treatment by subarea interaction ($P = 0.049$), the mean log-transformed count of DENV-infected *Ae. aegypti* females was compared between ITC and NTC homes within each of the two study subareas. The geometric mean count of DENV-infected *Ae. aegypti* females among NTC homes was six times larger than that for ITC homes in the East (0.024 and 0.004, respectively; $P = 0.040$), whereas there was no significant difference between NTC and ITC homes in the South ($P = 0.757$).

When also accounting for curtain use in the analysis, we found that the interaction of treatment by CUI was statistically significant for count of DENV-infected *Ae. aegypti* females for South and East combined ($P = 0.0495$). In particular, based on least significant difference pairwise mean comparisons, the geometric mean count of DENV-infected *Ae. aegypti* females for ITC homes (0.00) was significantly less than that for NTC homes (0.02 per month per house – equivalently, 0.23 per house during the study period) in the fourth quartile of household CUI ($P = 0.035$); the mean log-transformed count of DENV-infected *Ae. aegypti* did not differ significantly between NTC and ITC homes for the other three quartiles of household CUI (Table 4). Combining South and East in this particular comparison was justified because the second-order interaction of treatment, area, and CUI was not statistically significant ($P = 0.195$).

Virologic outcomes for humans. Key virologic outcomes for humans, including overall DENV infection, presence of multiple infected individuals within single homes and clustering of human infections, are described below.

Overall DENV infection. At baseline, there were no significant differences with respect to short-term DENV exposure in the months leading up to the study, based on IgM-capture enzyme-linked immunosorbent assay, for participants living

TABLE 4

Abundance of dengue virus-infected *Aedes aegypti* females in homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) by Curtain Use Index (CUI) for subareas south and east combined in Mérida, Mexico

CUI quartile*	Geometric mean count of dengue virus-infected <i>Ae. aegypti</i> females per home during the study period (total no. infected females collected)			
	NTC homes		ITC homes	
	No. homes included	Geometric mean count	No. homes included	Geometric mean count
1st–3rd quartile (limited, low, and medium use)	132	0.13 (34)	129	0.09 (16)
4th quartile (high use)	47	0.23† (16)	39	0.00† (0)

* 1st–3rd quartile, mean CUI = 0–0.339; 4th quartile, CUI = 0.340–1.

† Significant difference between NTC and ITC homes ($P = 0.035$).

TABLE 5

Distribution of single vs. multiple dengue virus (DENV) infections in humans in homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) in south or east study subareas in Mérida, Mexico

DENV infections in humans by individual home	Percentage of homes with single or multiple DENV infections in humans	
	NTC homes	ITC homes
Single infection	51.3 (40/78)	48.7 (38/78)
Multiple infection	70.6* (24/34)	29.4 (10/34)

* Significantly greater than an equal split (i.e., 50%) between NTC and ITC homes ($P = 0.016$).

in ITC versus NTC homes for either the East subarea (0%, 0 of 71 and 0.9%, 3 of 325, respectively) or the South subarea (0.3%, 2 of 723 and 0.2%, 1 of 465, respectively) (Table 2). For the full post-curtain installation period, the infection prevalence in the East for individuals residing in ITC homes (18.6%, 16 of 86) tended to be lower than for individuals in NTC homes (24.2%, 76 of 314), but the difference was not statistically significant ($P = 0.274$) (Table 2). No similar trend was seen in the South for ITC homes (7.1%, 45 of 636) versus NTC homes (7.3%, 30 of 413).

Homes with multiple human DENV infections. Post-curtain installation, human infections were detected in participants of 112 (32.0%) of the homes. Of these homes, 78 had a single person with evidence of DENV infection, and 34 homes had multiple-infected persons (Table 5). Homes with single infections were distributed evenly across NTC homes (51.3%, 40 of 78) and ITC homes (48.7%, 38 of 78). Extradomicillary DENV transmission likely contributed significantly to these infections. However, homes with multiple DENV infected persons deviated from the expectation of being evenly distributed across NTC and ITC homes: 70.6% (24 of 34) among NTC homes compared with 29.4% (10 of 34) among ITC homes ($P = 0.016$; Table 5). This finding strongly suggests that the presence of ITCs reduces intradomicillary DENV transmission.

Multiple logistic regression analysis for clustering of human infections. Data on evidence of human DENV infection taken either before curtain installation (including long-term exposures preceding the study) or post-curtain installation during study period 1 (September–December 2009), period 2 (January–July 2010), or periods 1 and 2 combined were obtained from as many members of each study household as possible (Table 6). At baseline, the odds of a DENV-infected person was significantly

TABLE 6

Evidence of human dengue virus (DENV) infection by time period for subareas south and east combined in Mérida, Mexico

Time period*	No. homes examined	No. persons examined	No. DENV infections (% positive)
All homes			
Baseline	370	1,593	396 (24.9)
Study period 1	346	1,373	133 (9.7)
Study period 2	256	674	37 (5.5)
Study periods 1–2	350	1,449	167 (11.5)
Homes from which data for Curtain Use Index were available			
Baseline	320	1,396	371 (26.6)
Study period 1	316	1,282	126 (9.8)
Study period 2	244	649	36 (5.5)
Study periods 1–2	319	1,353	159 (11.8)

* Based on pre-curtain installation baseline samples taken during May–September 2009 and post-curtain installation samples for study periods 1 and 2 taken during November 2009–January 2010 and July–August 2010, respectively.

greater for those residing in the East subarea compared with the South subarea ($P < 0.001$) but was not significantly different between ITC and NTC homes ($P = 0.419$) and there was no significant treatment by subarea interaction ($P = 0.314$). The results for study period 1 (September–December 2009) were similar to those for the baseline, namely, a significantly greater odds of a DENV-infected person in the East compared with the South ($P < 0.001$), and no significant difference between ITC and NTC homes ($P = 0.679$) or a significant treatment by subarea interaction ($P = 0.304$). Probably because of a limited number of DENV infections occurring during study period 2 (January–July 2010), there was no statistically significant difference between East and South ($P = 0.562$) or between ITC and NTC homes ($P = 0.253$), or for the treatment by subarea interaction ($P = 0.897$). Consequently, the results for the full study period 1–2 were similar to those seen for study period 1, namely, a significantly greater odds of a DENV-infected person in the East compared with the South ($P < 0.001$), no significant difference between ITC and NTC homes ($P = 0.431$), and no significant treatment by subarea interaction ($P = 0.498$).

Because data for CUI were not available for all study homes, analyses of data with regards to CUI were based on reduced numbers of sampled houses and persons and, in general, a reduced number of persons with evidence of dengue virus infection (Table 6). The addition of the CUI as a main effect in the analysis model did not change the outcome of the analyses described above; most notably, there was no evidence of high CUI being protective in ITC homes as observed for infections in mosquitoes.

Age-specific DENV infection prevalence. Post-curtain installation, there were trends for the younger age groups (< 5 and 5–18 years of age) toward reduced prevalence of DENV-infection in ITC homes in the East (Table 7). No similar trends were seen for these age groups in the South, or for adults in either the East or South.

Entomologic outcomes. Key entomologic outcomes, including mosquito abundance, intradomicile use, blood feeding status and insecticide resistance profile, are described below.

Mosquito abundance in ITC versus NTC homes. No significant differences between ITC and NTC homes were recorded for abundance of *Ae. aegypti* females before the curtains were installed. Post-curtain installation, based on repeated-measures ANOVA of log-transformed counts – specifically, $\log_{10}(\text{count} + 1)$ because of the presence of zero counts, the count of *Ae. aegypti* females did not differ significantly either between ITC and NTC homes or between the two study subareas without any significant treatment by subarea interaction ($P = 0.978$, $P = 0.849$, and $P = 0.593$, respectively). However, as might be expected, the count of *Ae. aegypti* females did vary significantly by month of collection

($P < 0.001$). Because of the statistically significant interaction between treatment and collection month ($P = 0.028$), the mean log-transformed count of *Ae. aegypti* females was compared between ITC and NTC homes for each of the collection months based on Fisher's least significant difference method. Although the geometric mean count of *Ae. aegypti* females did not differ significantly for NTC versus ITC homes for any of the sampled months, it was 27% lower for ITC homes compared with NTC homes for the first post-curtain installation collection in September (1.96 and 2.65, respectively). Notably, four ITC homes yielded very high total numbers (range = 146–397) of *Ae. aegypti* females during the study, as compared with two NTC homes (range = 151–178). Further examination of these premises revealed potential factors that could have led to high mosquito infestation, including the presence of unusual but potentially productive larval development sites (e.g., rain water collection systems) next to the homes and inappropriate curtain use.

No significant differences between ITC and NTC homes were recorded for abundance of *Cx. quinquefasciatus* females before the curtains were installed. Post-curtain installation, the log-transformed count of *Cx. quinquefasciatus* females differed significantly between the South and East subareas but not between ITC and NTC homes without any significant treatment by subarea interaction ($P < 0.001$, $P = 0.178$, and $P = 0.279$, respectively). The mean count of *Cx. quinquefasciatus* females varied significantly by collection month post-curtain installation without significant interaction between treatment and collection month interaction ($P < 0.001$ and $P = 0.520$, respectively). Because of the statistically significant treatment by subarea by collection month interaction ($P = 0.034$), the mean log-transformed count of *Cx. quinquefasciatus* females was compared between ITC and NTC homes within each of the two study subareas for each of the post-curtain installation monthly collections. In the South, the geometric mean count of *Cx. quinquefasciatus* females for ITC homes was significantly less than that for NTC homes for 7 of the 11 months (September, November, and December 2009, and January, February, May, and July 2010). In the East, there were no significant differences between ITC and NTC homes for any of the post-curtain installation collection months. The trend towards lower abundance of females in ITC homes in the South is opposite of the *Ae. aegypti* results. We are currently trying to discern which aspects of the biology of *Cx. quinquefasciatus*, relative to *Ae. aegypti*, that might account for the difference between these two species.

Overall, the ANOVA of log-transformed counts of mosquito females found the significance or non-significance of the main effects for subarea, treatment, and collection month, as well as their interactions, to be essentially unaffected by including

TABLE 7

Dengue virus (DENV) exposure of humans by age group in homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) in south or east study subareas in Mérida, Mexico

Age group, years	Post-curtain installation evidence of DENV exposure							
	East subarea				South subarea			
	NTC homes		ITC homes		NTC homes		ITC homes	
	No. examined	Percentage (no.) with evidence of DENV exposure	No. examined	Percentage (no.) with evidence of DENV exposure	No. examined	Percentage (no.) with evidence of DENV exposure	No. examined	Percentage (no.) with evidence of DENV exposure
< 5	28	10.7 (3)	6	0.0 (0)	39	0.0 (0)	73	0.0 (0)
5–18	125	23.2 (29)	36	11.1 (4)	157	5.7 (9)	206	5.3 (11)
> 18	161	27.3 (44)	44	27.3 (12)	213	9.9 (21)	355	9.6 (34)

TABLE 8

Blood feeding status of *Aedes aegypti* and *Culex quinquefasciatus* females collected from homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) by study period for subareas south and east combined in Mérida, Mexico

Time period*	<i>Ae. aegypti</i> females			<i>Cx. quinquefasciatus</i> females		
	Total no. examined for blood feeding status	Percentage (no.) unfed	Percentage (no.) fed	Total no. examined for blood feeding status	Percentage (no.) unfed	Percentage (no.) fed
NTC homes						
Study period 1	1,982	24.5% (485)	75.5% (1,497)	2,527	15.7% (397)	84.3† (2,130)
Study period 2	2,396	23.2% (557)	76.8% (1,839)	4,274	20.4% (871)	79.6† (3,403)
ITC homes						
Study period 1	2,068	28.3% (586)	71.7%† (1,482)	1,637	17.3% (284)	82.7† (1,353)
Study period 2	2,136	16.1% (343)	83.9%† (1,793)	2,991	14.0% (419)	86.0† (2,572)

*Study period 1 = September–December 2009; study period 2 = January–July 2010.

†Significant difference between study period 1 and study period 2 for NTC and ITC homes ($P \leq 0.003$).

the CUI, defined as quartiles of household CUI, as a main effect in the ANOVA models for either *Ae. aegypti* females ($P = 0.124$) or *Cx. quinquefasciatus* females ($P = 0.748$).

Correlation between abundance of *Ae. aegypti* and *Cx. quinquefasciatus* in study homes. As noted above, certain homes were extraordinarily permissive to infestation with *Ae. aegypti*. Many of these same homes were also permissive to infestation with *Cx. quinquefasciatus*. Homes that were examined on all 11 possible post-curtain installation sampling occasions were used to determine the strength of the correlation between overall numbers of collected *Ae. aegypti* and *Cx. quinquefasciatus* (females and males combined). This demonstrated significant positive correlations between total numbers of collected *Ae. aegypti* and *Cx. quinquefasciatus* in NTC homes (Spearman's rank correlation $\rho = 0.368$, $n = 86$, $P < 0.001$), as well as in ITC homes ($\rho = 0.513$, $n = 77$, $P < 0.001$). The factors that make certain homes permissive to both of these species remain to be determined, and understanding these factors could be very important in targeting control efforts for these vector species.

Mosquito feeding success in ITC and NTC homes. Even if a mosquito survives contact with an ITC, its physiological functions, such as the ability to successfully locate a human or take a full blood meal, could be impacted for some period of time. On the suspicion that sub-lethal ITC contact may temporarily result in biting or feeding inhibition, we compared the feeding status of *Ae. aegypti* females collected post-curtain installation in and around ITC versus NTC homes. Intriguingly, the percentage of blood-fed females was consistent across study periods 1 and 2 for NTC homes ($P = 0.344$) but increased from study period 1 through 2 for all ITC homes ($P < 0.001$) (Table 8). This increase in the proportion of fed females during the latter study period in ITC homes could be associated with the concurrent reduction in deltamethrin concentration in the ITCs, which could have resulted in reduced biting inhibition from study period 1 through 2. The same type of

pattern was observed for *Cx. quinquefasciatus* females (Table 8), for which a significant decrease in the percentage of blood fed females from study period 1 through 2 in NTC homes ($P < 0.001$) contrasted with a significant increase from study period 1 through 2 in ITC homes ($P = 0.003$).

Mosquito collection by room type. Confirming previous results on collection of *Ae. aegypti* females by room type in Mérida,⁹ we found that most of the specimens collected indoors after the curtains were installed and for which the room type of collection was known ($n = 8,714$) came from bedrooms (59.6%) followed by living/dining rooms (21.8%). When broken down by NTC versus ITC home, we found that bedrooms in ITC homes accounted for a greater proportion of collected *Ae. aegypti* females (63.5%, 2,754 of 4,340) compared with NTC homes (55.8%, 2,442 of 4,374) ($P < 0.001$). Similar results were recorded for *Cx. quinquefasciatus* females, with most of the specimens ($n = 11,663$) collected from bedrooms (52.2%) followed by living/dining rooms (19.3%). Bedrooms in ITC homes accounted for a greater proportion of collected *Cx. quinquefasciatus* females (59.3%, 2,903 of 4,899) compared with NTC homes (47.1%, 3,185 of 6,764) ($P < 0.001$).

Knock-down resistance allele in *Ae. aegypti*. The knock-down resistance-conferring allele Ile 1,016 was monitored in *Ae. aegypti* mosquitoes collected from homes with ITCs and NTCs at the beginning and end of the study to determine if intrusion into ITC homes was related to the presence of this allele. There were no significant differences in frequencies of mosquitoes with the Ile 1,016 allele between ITC and NTC homes at either the start or the end of the study (Table 9). There was no increase in frequency of the Ile 1,016 allele from the start to the end of the study in the ITC homes.

Protective measures used by study participants to kill mosquitoes inside their homes. Study participants were interviewed to determine if they were using measures to control mosquitoes in the homes (Table 10). Approximately 70% of participants reported using spray cans with insecticides to control

TABLE 9

Detection of the knock-down resistance-conferring Ile 1,016 allele in *Aedes aegypti* collected from homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) for subareas south and east combined in Mérida, Mexico*

Type of home	<i>Ae. aegypti</i> collected pre-curtain installation						<i>Ae. aegypti</i> collected post-curtain installation					
	No. examined	AA	AG	GG	% AA	Frequency of A	No. examined	AA	AG	GG	% AA	Frequency of A
NTC	65	43	19	3	66.2	0.81	532	308	174	50	57.9	0.74
ITC	70	57	12	1	81.4	0.90	695	419	214	62	60.3	0.76
All	135	100	31	4	74.1	0.86	1,227	727	388	112	59.3	0.75

*AA = Ile 1,016 homozygotes; AG = Ile 1,016/Val 1,016 heterozygotes; GG = Val 1,016 homozygotes.

TABLE 10

Home owner usage of mosquito control measures during the study in homes equipped with insecticide-treated window curtains (ITCs) vs. non-treated window curtains (NTCs) for subareas south and east combined in Mérida, Mexico

Control measure	NTC homes	ITC homes
Taking action to kill mosquitoes inside the house		
Yes	69.9% (95/136)	70.9% (90/127)
No	30.1% (41/136)	29.1% (37/127)
Methods used to kill mosquitoes inside the house		
Spray cans with insecticide	75.7% (78/103)	68.5% (63/92)
Mosquito coils with insecticide	9.7% (10/103)	9.8% (9/92)
Electric plug-in emitting insecticide or repellent	9.7% (10/103)	19.6% (18/92)
Other method	4.9% (5/103)	2.2% (2/92)
How often are these mosquito killing methods used		
Every day	23.1% (24/104)	27.9% (31/111)
Every week	23.1% (24/104)	13.5% (15/111)
Sometimes	42.3% (44/104)	36.0% (40/111)
Never	8.7% (9/104)	21.6% (24/111)
Not sure	2.9% (3/104)	0.9% (1/111)

mosquitoes in the home. In addition, 10–20% reported using insecticide emitters for mosquito control.

DISCUSSION

Recent studies demonstrated the potential for use of ITCs to protect homes from *Ae. aegypti* infestation and to reduce DENV infections in humans in a public health community-based intervention in which most or all of the homes in an intervention area received ITCs.^{13,19,20} This type of broad-scale intervention appears to provide a community effect in which non-treated homes may also be protected.¹³ In this study, we instead wanted to determine if individual homeowners can use ITCs to prevent intrusion by *Ae. aegypti* and to reduce the risk for intradomicillary DENV transmission within their own homes. We therefore designated this study a consumer product intervention, in which homes equipped with ITCs and NTCs were located at a minimum distance from each other that exceeds the typical flight range of *Ae. aegypti* to preclude a community effect of the ITC intervention. The study focused on closed concrete housing, which is common throughout the developing world and for which new home-targeted control measures are critical because traditional vehicle-based space spraying of insecticides is ineffective in penetrating into this type of home, which thus provides a refugium for the endophilic *Ae. aegypti*.^{36–38} Our study yielded information concerning best practices for using ITCs to prevent intradomicillary DENV transmission.

Overall in 2009, there were 2,028 diagnosed dengue cases reported from the city of Mérida. The level of DENV activity varied among neighborhoods of the city and so did the intensity of vector control efforts. Our two study subareas, designated South and East, differed with regards to DENV transmission intensity in 2009; both mosquito infections and human infections were more prevalent in NTC homes in the East compared with the South. These subareas also differed for virologic and entomologic outcomes of the *Casa Segura* intervention. In the East, the number of human infections was lower, albeit not significantly lower, and the number of DENV-infected

Ae. aegypti females was significantly reduced in ITC versus NTC homes. The results were not the same in the South, in which the number of DENV infected humans or infected mosquitoes did not differ significantly between ITC and NTC homes. The reasons for this difference remain to be determined. One contributing factor may have been that DENV transmission was more intense in the East than in the South, which makes it easier to statistically demonstrate a reduction in infection prevalence in an intervention study.

Another potential explanation is ITCs being used more appropriately in the East compared with the South during the peak of the DENV transmission season in the fall of 2009 (prior to when we started measuring the curtain use index). Servicios de Salud de Yucatán follows national guidelines for vector control and responds to reported dengue outbreaks with vehicle and backpack ultra-low volume spraying and larval control in and around homes or areas with suspected dengue cases. Because the East had many more cases than the South in the fall of 2009, the public health vector control activities and publicity surrounding the outbreak may have prompted better curtain use and thus better protection of ITC homes. Appropriate use of the ITCs leads to reduced indoor numbers of DENV-infected *Ae. aegypti* females; not a single infected mosquito was detected from the homes falling in the highest quartile for appropriate curtain use (Table 4). Alternatively, better curtain use may have driven vectors from the indoor environment to the outside of homes, where they would be much more vulnerable to space spraying. We also note that in general, study homes in the South were older and patios had more foliage than those in the East, which may have impacted the production of mosquitoes and their ability to penetrate the homes, thereby reducing the protective efficacy of the ITCs. The more abundant foliage and the reduced Servicios de Salud de Yucatán control efforts in the South may have provided a more hospitable environment outside the homes for vectors and perhaps DENV transmission could occur outside of the domicile. Clearly, more information is required to understand factors that conditioned the efficacy of the *Casa Segura* in the East but not the South and to optimize *Ae. aegypti* control in and around the home.

The presence of ITCs tended to reduce *Ae. aegypti* females in the homes for a short period of time after their installation and *Cx. quinquefasciatus* females throughout much of the study period. Perhaps the most intriguing finding in the study was that homes where the ITCs were used most appropriately still contained *Ae. aegypti* females but significantly reduced numbers of DENV-infected *Ae. aegypti* females. This finding could be attributable to a combination of factors resulting from sub-lethal exposure of mosquitoes to deltamethrin-treated curtains, potentially including 1) biting inhibition, as suggested by an increased proportion of fed *Ae. aegypti* females in ITC homes during study period 2 when the deltamethrin concentration of the ITCs was decreasing (Table 8); 2) reduced lifespan of mosquitoes after sub-lethal ITC contact; and 3) repellency, which would promote exophagy and feeding on vertebrates other than humans. All of these factors could reduce indoor DENV transmission and the prevalence of DENV-infected mosquitoes. As a case in point, a recent study showed that sub-lethal topical exposure of *Ae. aegypti* and *Cx. quinquefasciatus* females to pyrethroids, such as deltamethrin and permethrin, results in temporary impairment of flight activity and reduced movement towards attractants such as carbon dioxide.³⁹

Deltamethrin concentration in ITCs decreased during the study; ITC samples assayed at baseline (May to June 2009) contained an average of 2.56 g/kg of deltamethrin, whereas samples assayed at the end of the study (June 2010) contained an average of 2.07 g/kg of deltamethrin. Our understanding of potential sub-lethal effects on *Ae. aegypti* (e.g., reduced longevity or biting inhibition) induced by proximity to or contact with a deltamethrin-treated textile in the closed concrete housing examined in our study is extremely limited. These issues would seem to be fruitful areas of future research.

There was a positive correlation between total numbers of collected *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes in the study homes. This finding indicates that homes that were permissive to infestation with *Ae. aegypti* also were permissive to *Cx. quinquefasciatus*, and suggests that the mosquitoes may exploit the same entry points into a home. Most of the females collected indoors were blood fed, suggesting that indoor feeding is a common occurrence for both species. Numerous homes in the study produced large numbers of *Ae. aegypti* females after the curtains were installed: 36 homes yielded > 50 *Ae. aegypti* females and six super-infested households, including four ITC homes, yielded totals of 146–397 *Ae. aegypti* females. Interestingly, every member of these super-infested homes either had antibody to DENV or seroconverted during the study. Further examination of these premises showed potential factors that could have led to this high mosquito infestation, including presence of unusual and potentially productive larval development sites (e.g., rain water collection systems), major accumulations of water-holding containers, and potentially non-compliant curtain use. Two of the ITC super-infested homes had low CUIs but two had high CUIs. It is noteworthy that the two ITC homes with higher CUIs had dramatically lower numbers of mosquitoes after the CUI index monitoring was instituted. Whether the monitoring increased appropriate curtain use is not known. Clearly however, future ITC-based interventions should vigorously promote, and if possible, monitor, appropriate curtain use.

We speculate that abundant larval development sites around a home can result in mosquito abundances of such a magnitude that the protective effect of the *Casa Segura* is overwhelmed by the sheer numbers of mosquitoes attempting to enter homes equipped with ITCs. For example, they could enter a home while ITCs briefly are tied up or even while air movement temporarily moves an ITC out of position. These issues could be counteracted by an integrated management strategy combining the use of ITCs with source reduction efforts, such as México's *Patio Limpio* program.⁴⁰ Furthermore, intrusion into ITC homes may have resulted from door openings that were not equipped with either treated or non-treated textiles in this study. If the presence of ITCs in windows fails to make the home unpleasant enough for the mosquito to avoid entering, then unprotected doorways may become a major entry point into the home. In an ongoing *Casa Segura* study in Mexico, windows and doors of homes are being treated with curtains, which should enhance the protective efficacy of the approach.

The predominant collection of *Ae. aegypti* females from bedrooms confirms our previous results from Mérida,⁹ and here we show that *Cx. quinquefasciatus* females also were collected most commonly from bedrooms. *Aedes aegypti* is primarily a daytime feeder, and it would seem that infestation of bedrooms therefore would not be so important. However, the bedroom is where sick persons are, where friends and

family come to visit them, and where the mosquito vector often rests and presumably also feeds if given the opportunity. We consider exclusion of mosquito vectors from this epidemiologically significant room type to be of critical importance to the *Casa Segura* intervention, especially in light of our data showing that bedrooms in ITC homes accounted for a greater proportion of collected *Ae. aegypti* females (63.5%) compared with NTC homes (55.8%), with a similar, albeit non-significant, trend seen for DENV-infected *Ae. aegypti* females. This finding emphasizes the need to specifically target bedrooms for control efforts. Female mosquitoes likely are attracted to bedrooms because they are quiet areas of the home, contain attractive clothing and bedding, and often are located in the coolest and darkest part of the home towards the back. Furthermore, space spraying of insecticides, which is intensively used in Mérida to prevent and control dengue outbreaks, may only reach the front patio and front rooms, and thus have minimal impact in bedrooms located to the back of the home (García-Rejón JE, personal observation). More effective indoor control strategies need to specifically address the bedroom in addition to the other areas of the home.

The *Casa Segura* approach used herein exploited deltamethrin-treated window curtains to prevent *Ae. aegypti* from transmitting DENV in the home. The efficacy of the *Casa Segura* intervention and of pyrethroid-based space spraying for control of *Ae. aegypti* adults is predicated upon operational effectiveness of pyrethroid insecticides. Knock-down resistance to pyrethroids has emerged in *Ae. aegypti* in many parts of the tropical world including Mexico, and a rapidly increasing frequency of the knock-down resistance-conferring Ile 1,016 allele in *Ae. aegypti* in Mérida over the past decade is of great concern.^{35,41–45} However, little is known about the extent to which this factor may adversely affect operational vector control or which approaches could be used to mitigate the evolution of knock-down resistance. We found no evidence for the use of ITCs in individual households promoting knock-down resistance (Table 9), but this needs to be investigated in a community-based intervention in which all or most homes in an area receive ITCs. We are currently instituting an intervention in Mérida to address this issue. Furthermore, our use of deltamethrin-impregnated ITCs reduced DENV infection in *Ae. aegypti* females despite the intervention being implemented in areas with high frequencies of the knock-down resistance-conferring Ile 1,016 allele.

One intriguing finding was the extensive use of pyrethroid-based consumer products, especially spray cans, to control mosquitoes in the study homes (Table 10). This finding demonstrates a robust market for consumer products to control mosquitoes in the home in dengue-endemic areas. Our finding also suggests that in Mérida, *Ae. aegypti* is commonly exposed to pyrethroid-based insecticides both indoors (via consumer products) and outdoors (large-scale space spraying by the vector control program). The lack of either an indoor or an outdoor refugium for the mosquito with respect to use of pyrethroid-based insecticides may help to explain the rapid increase in knock-down resistance of *Ae. aegypti* observed in Mérida over the past decade. We further speculate that increased use of indoor pyrethroid-based consumer products, particularly a combination of ITCs and other products that specifically target the mosquitoes that penetrate the ITC barrier, may promote exophagy and exophily in *Ae. aegypti* and thus force the mosquito to spend more time in less protected outdoor environments. Increased time spent

outdoors could potentially decrease the likelihood of an infected mosquito surviving the extrinsic incubation period for DENV and reduce overall DENV transmission intensity.

Other intriguing findings included trends toward reduced DENV infections in children, especially those < 5 years of age, compared with adults in both subareas for ITC homes, as well as NTC homes, and toward reduced DENV infections in children in ITC homes versus NTC homes in the East (Table 7). In contrast, there was no difference in DENV infections in adult participants (> 18 years of age) residing in ITC versus NTC homes in the East, suggesting that DENV infections in older cohorts commonly resulted from extradomicillary virus transmission, for example in workplaces and other indoor environments. In this regard, 112 homes in the study experienced DENV infections; 36 of these homes experienced multiple DENV infections (Table 5). Multiple DENV infections were more frequently detected in NTC than in ITC homes (71% and 29%, respectively). In contrast single DENV infections were evenly distributed among NTC and ITC homes. We speculate that some of these single infections reflect study participants who were most likely infected through extradomicillary transmission of DENV. Multiple infections more likely reflect intradomicillary DENV transmission, which is mitigated by the presence of ITCs. In addition, multiple infected mosquitoes were detected in four ITC homes (average = 2.5 and range = 2–3 infected mosquitoes per home) and in five NTC homes (average = 6.2 and range = 2–9 infected mosquitoes per home). We conclude that the presence of the ITCs seems to reduce intradomicillary virus transmission.

Experimentally demonstrating the effectiveness of a vector control intervention is a difficult undertaking, especially against a backdrop of ongoing control activities that are independent of the experimental design and addresses diseases, such as dengue, that have unpredictable coverage in space and time. Because the intensity of DENV transmission varies between years, intervention studies may need to include multiple transmission seasons simply to achieve sufficient dengue case numbers and statistical power to evaluate the outcomes. Virologic outcomes based on DENV infections in humans are problematic because of the mobility of the population in urban areas; the site of virus exposure could be outside of the intervention area and is frequently difficult to identify.^{46–50} In our specific case with ITCs in individual homes, the outcome for infection in humans can be skewed by extradomicillary DENV exposures occurring in any environment outside of the participants' own homes, including homes of neighbors, friends and family, schools or workplaces and markets. Obtaining adequate numbers of participants in very young and potentially less mobile age groups (< 5 years of age) is frequently difficult, and they can also be infected outside of the home if that is the focus of the intervention.⁵¹ As a consequence, we propose that for *Ae. aegypti* control interventions, DENV-infected mosquitoes collected in and around the home may be a more appropriate metric for assessing control efficacy than DENV infections in humans. Because *Ae. aegypti* females are primarily endophilic and have a limited flight range,^{24,52} they are likely a better indicator of intradomicillary DENV transmission than even seroconversion in young children, who could be infected in other homes or elsewhere. There is no question that presence of DENV-infected vectors in the home is a major risk factor for transmission to still susceptible residents or visiting family and friends. In our study, several homes had multiple DENV-infected mosquitoes, and a few NTC homes produced as many as seven or nine infected

mosquitoes. Moreover, evidence is accumulating from Latin America that collection of DENV-infected *Ae. aegypti* females is feasible, and thus can serve as the basis for viable virological outcome measures in intervention studies.^{9,22,53–58}

In conclusion, the longevity of *Ae. aegypti*, its penchant for feeding frequently on humans even in a single gonotrophic cycle, and its preference for feeding on humans in domiciles and in bedrooms where DENV-infected humans typically are cared for and visited by friends and relatives make it a public health imperative to control the mosquito in the home and other indoor environments. Combining the *Casa Segura* approach for control of adult mosquitoes in the home with control of larval development sites in the peridomestic environment, for example through the *Patio Limpio* initiative in Mexico, will increase the protective efficacy of both approaches. We conclude that use of ITCs holds promise to protect against indoor DENV transmission, even in an area in which knock-down resistance to the pyrethroid used in the ITC occurs in *Ae. aegypti*, but also note that additional novel control methods for indoor use are needed to eliminate the mosquitoes that still penetrate an ITC barrier.

Received December 20, 2012. Accepted for publication April 16, 2013.

Published online June 3, 2013.

Dedication: This study is dedicated to the memory of Dr. Jose Trapaga-Barrientos of Bayer Environmental Science, México City, Mexico.

Acknowledgments: This study was administered by the Fundación de la Universidad Autónoma de Yucatán, A. C. We thank Mildred López, María Puc, Carlos Estrella, Alex Ic, Roger Arana, Wilberth Chi, Hugo Valenzuela, Iván Villanueva, Jesús Miss, Luis Flores, Rosa Cetina, Lourdes Talavera, Jorge Morales, Roger Lopez, Elsy Rosado, Nubia Rivero, Yamili Chan, Fernando Nieves, Daniela Flores, Ana Uitz, Jesus Baak, Fernando Chan, Claudia Carrillo, Lucio Cabrera, Guadalupe Arevalo, Eric Diaz, Rita Pacho, Luis Solis, Chac Sosa, Shijani Aguilar, and Marilia Caamal (Universidad Autónoma de Yucatán) for technical assistance with field and laboratory work; and the involved home owners and families for participating in this study, granting us permission to collect mosquitoes in their homes, and providing opinions and suggestions on *Casa Segura* products and interventions. We also thank Gerhard Hesse (Bayer Environmental Science, Lyon, France) for providing invaluable comments and suggestions and support of the study. The treated and non-treated textiles used in the study were kindly provided by Bayer de México S.A. de C.V., Bayer Environmental Science, México City, Mexico, and Acytex Internacional, México City, Mexico.

Financial support: The study was supported by the Innovative Vector Control Consortium and by the National Institutes of Health/ National Institute of Allergy and Infectious Diseases (International Collaborations in Infectious Disease Research Program U01-AI-088647).

Disclaimer: The contents of this study are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Disclosure: Some of the authors are employed by Bayer de México, one of the providers of the insecticide-treated curtains used in the study.

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