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Field evaluation of a lethal ovitrap against dengue vectors in Brazil

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Abstract. Field evaluation of a 'lethal ovitrap' (LO) to control dengue vector *Aedes* mosquitoes (Diptera: Culicidae), was undertaken in two Brazilian municipalities, Areia Branca and Nilopolis, in the State of Rio de Janeiro. The LO is designed to kill *Aedes* via an insecticide-treated ovistrip (impregnated with deltamethrin). In each municipality, the intervention was applied to a group of 30 houses (10 LOs/house) and compared to 30 houses without LOs in the same neighbourhood. Five LOs were put outside and five LOs inside each treated house.

Three methods of monitoring *Aedes* density were employed: (i) percentage of containers positive for larvae and/or pupae; (ii) total pupae/house; (iii) total adult females/house collected by aspirator indoors. Weekly mosquito surveys began during the month before LO placement, by sampling from different groups of 10 houses/week for 3 weeks pre-intervention (i.e. 30 houses/month) and for 3 months post-intervention in both treated and untreated areas.

Prior to LO placement at the end of February 2001, *Aedes aegypti* (L) densities were similar among houses scheduled for LO treatment and comparison (untreated control) at each municipality. Very few *Ae. albopictus* (Skuse) were found and this species was excluded from the assessment. Post-intervention densities of *Ae. aegypti* were significantly reduced for most comparators ($P < 0.01$), as shown by fewer positive containers (4–5 vs. 10–18) and pupae/house (0.3–0.7 vs. 8–10) at LO-treated vs. untreated houses, 3 months post-treatment at both municipalities. Numbers of adult *Ae. aegypti* females indoors were consistently reduced in LO-treated houses at Areia Branca (3.6 vs. 6.8/house 3 months post-intervention) but not at Niloplis (~3/house, attributed to immigration). These results demonstrate sustained impact of LOs on dengue vector population densities in housing conditions of Brazilian municipalities.

Key words. *Aedes aegypti*, *Aedes albopictus*, container breeding, dengue vectors, lethal ovitrap, mosquito control, pupa survey, Rio de Janeiro, Brazil.

Introduction

Dengue and dengue haemorrhagic fever (DHF) are mosquito-borne viral diseases coinciding with distribution of the yellow fever mosquito *Aedes aegypti*, the primary vector of dengue throughout the tropical and semitropical world (Gubler & Kuno, 1997). *Aedes aegypti* is an urban

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mosquito that has adapted to utilizing man-made containers (flower pots, small cisterns, discarded tyres and cans) for breeding, feeds primarily on humans (Christophers, 1960), and rests in secluded locations inside homes, e.g. under beds, in closets and on curtains (Perich *et al.*, 2000) where conventional insecticide treatments are minimally effective (Perich *et al.*, 1990). Within the last two decades *Aedes albopictus* (Skuse), a known vector of dengue in south-east Asia (Hawley, 1988), has been introduced to and spread throughout many areas of the Western Hemisphere, to include Brazil (PAHO, 1994). *Aedes albopictus* breeds in both man-made containers (e.g. cans, tyres, water jars, etc.) and in natural (e.g. bamboo, bromeliads, coconut shells, etc.) containers, is more cosmopolitan in its feeding habitats and rests both inside homes and outside, making control difficult.

Sustained control of *Ae. aegypti* and to some extent *Ae. albopictus* requires source reduction by environmental sanitation, as well as emergency insecticide treatment against the mosquitoes (PAHO, 1994). With environmental sanitation implementation, not all breeding sites can either be totally eliminated or made totally mosquito-proof and often not all individuals collaborate in clean-up campaigns. In addition, no adulticide or larvicide application against these dengue vectors is 100% effective. Thus, there is much opportunity and need for alternative control methods that are environmentally benign, cost-effective, and suitable for integration with control programs based on community participation. The development of a lethal ovitrap (LO) could be a practical, new method for control of *Ae. aegypti* and *Ae. albopictus*.

The ovitrap was first developed as a surveillance tool for *Ae. aegypti* in the United States (Fay & Perry, 1965; Fay & Eliason, 1966). It has been used in many parts of the world for detecting and monitoring dengue vector populations, especially for low density levels (Service, 1993). The ovitrap was first used for control of *Ae. aegypti* in 1969 at Singapore International Airport (Chan, 1973). Subsequently, Chan *et al.* (1977) designed an autocidal screened ovitrap that attracted more *Ae. aegypti* than other domestic container habitats in field tests. These studies demonstrated the feasibility of using a modified ovitrap for the control of dengue vectors. Therefore, we designed a lethal ovitrap (LO) that incorporated an insecticide-impregnated oviposition strip (ovistrip) and was found to significantly control *Ae. aegypti* in laboratory cage experiments (Zeichner & Perich, 1999). Therefore we proceeded to test the LO against the dengue vector populations in urban areas of Brazil.

Materials and methods

Study sites

Two municipalities, Nilopolis and Areia Branca, in the state of Rio de Janeiro, approximately 40 km south-east of Rio de Janeiro, Brazil were selected for test sites of the LO. Historically both municipalities have high populations of *Ae. aegypti*, and *Ae. albopictus* has been collected from both places by the Brazilian Ministry of Health (MOH) personnel. In addition, both municipalities are endemic with dengue as reported by the Brazilian MOH. Two neighbourhoods, a minimum of 1 km apart in each municipality were selected with one randomly chosen in each municipality to receive the LO treatment and the other to serve as the untreated control. Each neighbourhood was comprised of similar house type, one storey structures with a kitchen, living room, bathroom and 1–2 bedrooms. Most houses had a small front garden area and larger garden area at the back of the house. The two neighbourhoods in Nilopolis compared to the two in Areia Branca had substantially more non-serviceable containers (e.g. cans, bottles, used tyres, etc.), which were potential dengue vector breeding sites.

Sampling

Thirty houses at the two neighbourhoods in both Nilopolis and Areia Branca were selected to serve as the sample houses. A house was the unit of sample and comprised both the inside portion of the home and the front, side and rear outdoor areas of the house property. Pre-treatment sampling was initiated 3 weeks prior to placement of the LOs at all treatment and control neighbourhoods. Ten houses at each treatment and control site were sampled weekly and rotated each week between the 30 sample houses at each site. This was done to control for any possible vector population reduction due to the sampling methods. One week after placing the LOs at the treatment sites, post-treatment sampling was initiated and done weekly on a 10 house per site sample rotation for the next 3 months.

Three sampling procedures were used to measure the *Aedes* mosquito abundance: (i) the total number of containers per house containing mosquito larvae and/or pupae (positive container index), (ii) a pupal survey (mean number of mosquito pupae found per house) as described by Focks & Chadee (1997) and (iii) adult mosquito aspiration collection. The adult mosquito collections were done by two MOH persons using flashlights and battery-powered hand-held aspirators (Hausher Machine Works, Tomÿ's River, N.J., U.S.A.) collecting all mosquitoes within the house for 10 min. Mosquitoes collected were placed in a container labelled with date and house identification and returned to the laboratory for identification to species and number recorded.

In addition to the mosquito population sampling, the level of insecticide susceptibility in Nilopolis and Areia Branca dengue vector populations was determined prior to LO treatment and after. Ovitraps were placed at houses both in the treatment and control sites in both municipalities 1 week prior to pre-treatment sampling and again 1 week after the last post-treatment sampling. Oviposition strips were collected 3 days after putting out ovitraps, allowed to dry, labelled with site location and then sent to the Entomology Branch at the Center for Disease Control and Prevention (CDC) in Atlanta, Georgia, U.S.A. At the CDC, laboratory eggs were hatched, reared to adults and insecticide susceptibility determined by microplate and bottle bioassay (Brogdon & McAllister, 1998) were done to determine the level of pyrethroid susceptibility of the vector populations from the test sites before and after LO treatment.

Treatment

The lethal ovitrap (LO) used in the field tests in Brazil was the same black polyethylene cup, 473 ml capacity, with an 11 cm × 2.5 cm red velour heavy-weight paper strip serving as the ovistrip and attached to the cup with a paper clip as described in the prior laboratory testing of the LO (Zeichner & Perich, 1999). The ovistrips used in these field tests were treated with 1.0 mg a.i./strip of deltamethrin insecticide, the insecticide found to be most efficacious in the prior laboratory testing (Zeichner & Perich, 1999). The LO is a U.S. patented device (U.S. patent number 5,983,557, 11 November 1999) and has International patents filed.

To enhance the attractiveness of the LOs to the dengue vectors when placed in the field, the LOs were filled with a 10% hay infusion-water solution as described by Reiter *et al.* (1991) to approximately 2 cm of the top. Lethal ovitraps were placed at all 30 designated treatment homes at Nilopolis and Areia Branca. In addition, LOs were placed at all homes in the blocks surrounding the 30 treatment sample homes. This was done to control for migration of mosquitoes breeding in untreated homes into the treated areas that were sampled. Each house received five LOs placed outside the house in secluded sites out of direct sunlight. An additional five LOs were placed inside the house, with one in the kitchen, bathroom, living room and each bedroom, again in secluded sites. The LOs were checked weekly, hay infusion water added as needed, ovistrips replaced if found missing or entire LO replaced if found missing. All treated ovistrips were replaced every 30 days with fresh treated ovistrips for the 3 months of treatment.

Data analysis

The experimental design of the LO Brazilian field tests was a completely randomized design (Winer *et al.*, 1991). The significant difference between the mean number of positive containers for houses with the LO and those without was determined using a Chi-square test (StatExact, 1994). Significance for both mean pupal levels per house and mean number of adult mosquitoes collected per house was determined using a two-tailed *t*-test (StatExact, 1994).

Results and discussion

Both microplate and bottle bioassays determined that the *Ae. aegypti* populations of both Nilopolis and Areia Branca were 100% susceptible to pyrethroid insecticides before and after the LOs were placed out. This indicated that the deltamethrin, a pyrethroid insecticide used to treat the LO ovistrip, would be efficacious against the adult mosquitoes in the two Brazilian municipalities. In addition, the susceptibility testing after the completion of the 3 months of LO testing indicated that the LO did not adversely affect the pyrethroid insecticide susceptibility of the *Ae. aegypti* population at the test sites.

Only eight *Ae. albopictus* were collected from Nilopolis and only 14 from Areia Branca for the entire 4 months of this test. Therefore, they were not included in the data analysis due to such low numbers collected.

All three sampling measures (positive container index, mean pupal number per house, and adult mosquito aspiration collection) determined that the LO significantly affected the *Ae. aegypti* populations at the Brazilian test sites (Tables 1–3). The number of positive containers at Nilopolis and Areia Branca at both the designated treatment and untreated control blocks was not significantly different (Table 1 and Fig. 1a) prior to LO placement (pre-treatment), with a slightly greater number at both treatment blocks compared to the corresponding designated untreated control blocks. The LOs did effectively compete with the other domestic containers for potential *Ae. aegypti* oviposition as shown in the significant reduction in the number of positive containers (Fig. 1a); these results are similar to the results reported by Chan *et al.* (1977) in Singapore with their autocidal screened ovitrap. The reduction in our study occurred within 30 days after first putting the LOs in Areia Branca and took slightly longer in Nilopolis (Fig. 1a) where there was a greater number of non-serviceable containers, which served as larval/pupal habitats. Both sites over the 3 months of LO treatment had significantly ($P < 0.01$) fewer positive containers, 4–5 for the treated blocks compared to 10–18 for the untreated blocks (Table 1).

Similar results were found with the mean number of pupae per house, again with no significant difference between treatment and control houses in both municipalities (Table 2). Again, 30 days after the LOs were placed inside and outside the designated treatment houses, there was marked difference in the total number of pupae collected from those houses compared to the untreated control

Table 1. Proportions of containers positive for *Aedes aegypti* larvae and/or pupae (container index) per house in two municipalities, before and after intervention, comparing blocks with or without LO tratment (χ^2 test) as described in the text (c.f. Fig. 1a)

Municipality/ treatment	Mean % positive containers/house		
	Pre-treatment	Post-treatment	diff.
Nilopolis			
Untreated	4.8	10.0	$P < 0.01$
LO treated	6.1	5.1	
Areia Branca			
Untreated	13.2	17.6	$P < 0.01$
LO treated	10.3	3.6	

Table 2. *Aedes aegypti* pupal densities (number/house) in two municipalities, before and after intervention, comparing blocks with or without LO treatment as described in the text (c.f. Fig. 1b)

	Mean pupae/house		
Municipality/treatment	Pre-treatment	Post-treatment	*t*-test
Nilopolis			
Untreated	0.23	10.04	$P < 0.05$
LO treated	1.03	0.27	
Areia Branca			
Untreated	3.08	8.30	$P < 0.05$
LO treated	1.97	0.72	

houses, especially for Areia Branca where there were fewer competing breeding containers (Fig. 1b). After 60 days of LO treatment at both Nilopolis and Areia Branca the number of pupae at the treatment houses was reduced to 0 (Fig. 1b). The overall effect of the LO on the mean number of pupae per house was significant ($P < 0.05$) with a mean pupal number of 10 and 8.3 for the control blocks and only 0.27 and 0.72 for the treatment blocks at Nilopolis but, Areia Branca, respectively (Table 2). The pupal survey is a more accurate measure of dengue vector densities, particularly *Ae. aegypti* (Focks & Chadee, 1997), with this indices based on the last immature life stage before adulthood. Thus results in this study, based on pupal surveys, indicate that the LO had an adverse effect on the *Ae. aegypti* populations in the treatment blocks of both municipalities.

Female adult *Ae. aegypti* densities, as determined by house aspirations prior to LO placement, were found to be not significantly different between the treatment and control blocks at either municipality and the mean number collected per house (0.36–1.03) (Table 3). After LO distribution at the treatment houses in Areia Branca, the total number of female *Ae. aegypti* collected by aspiration from houses with the LOs was fewer then from the control houses (Fig. 1c). Sixty and 90 days after LO placement in Areia Branca, the number of female *Ae. aegypti* collected was significantly fewer from the treatment houses with only 24 and 10 mosquitoes aspirated after 60 and 90 days LO treatment, respectively, as compared to 62 and 61 collected from the control houses. This indicates that the LO over time significantly lowered adult female *Ae. aegypti*, the target population in Areia Branca. The overall mean number of female *Ae. aegypti* aspirated from the treatment houses (3.00) compared to the mean number collected from the

Table 3. Numbers of adult *Aedes aegypti* females/house in two municipalities, before and after intervention, comparing blocks with or without LO treatment as described in the text (c.f. Fig. 1c)

	Mean mosquitoes/house		
Municipality/treatment	Pre-treatment	Post-treatment	*t*-test
Nilopolis			
Untreated	0.53	2.67	$P = 0.45$
LO treated	0.70	3.00	
Areia Branca			
Untreated	0.36	6.83	$P < 0.05$
LO treated	1.03	3.59	

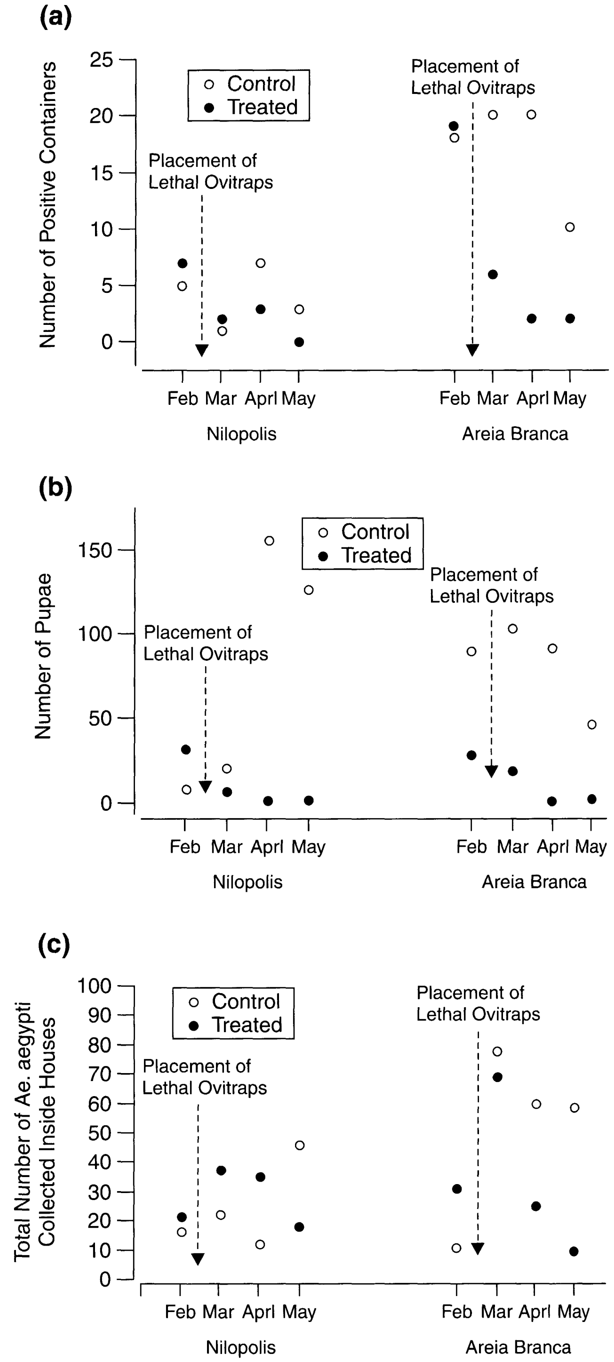

Fig. 1. Monthly mean densities of *Aedes aegypti* pre-intervention (February) and post-intervention (March–May, 2001), comparing sites treated (10 LOs/house) and untreated (without LOs) at Areia Branca and Nilopolis (10 houses/week/site). (a) Numbers of containers positive for larvae and/or pupae; (b) total pupae collected from all containers at 10 houses; (c) total adult females aspirated from 10 houses.

control houses (2.67) in Nilopolis (Table 3) was not significantly different and was actually slightly higher. This would at first indicate that the LO had no detrimental effect on the target adult *Ae. aegypti* population, but by the third month (May) of LO treatment in Nilopolis, we collected significantly fewer female *Ae. aegypti* (17) in the treatment houses compared to the control houses (48) (Fig. 1c). Nilopolis was the municipality with the greater number of non-serviceable containers (cans, bottles, used tyres, etc.) competing with the LO for the oviposition seeking *Ae. aegypti* and thus required a longer time period to reduce the overall adult *Ae. aegypti* population in Nilopolis.

Conclusions

We have demonstrated that in two municipalities near Rio de Janerio, Brazil, the lethal ovitrap (LO) (U.S. patent # 5,987,557, 16 November 1999) with an oviposition strip treated with 1.09 mg of deltamethrin significantly affected the natural populations of *Ae. aegypti*. The LO in Areia Branca, the municipality with the better sanitation programme (fewer non-serviceable containers), had significant impact on the *Ae. aegypti* population as measured by all three sampling parameters (positive container index, mean number of pupae/house, adult mosquito aspiration collection). In addition, the LO had a significant detrimental effect on the *Ae. aegypti* in Areia Branca 30 days after placing the LOs inside and outside the treatment houses. This indicates the potential of the LO to aid in the control of *Ae. aegypti* when integrated with community participation programs that target removal of non-serviceable containers, which can serve as breeding sites for this primary dengue vector. The effects of the LO in Nilopolis, the municipality with a greater number of non-serviceable containers, were overall significant as determined by the immature sampling procedures (positive container index and mean number of pupae/house), but required a longer treatment period (60 days post-placement of the LOs) to cause the detrimental effect on the *Ae. aegypti* population. The adult mosquito aspiration collection data from Nilopolis demonstrated that, for this municipality, the LO treatment required more time (90 days) compared to Areia Branca (<30 days) to cause a detrimental effect on the vector target population. Although the LO required a longer treatment time period to exert a significant effect against the *Ae. aegypti* in Nilopolis, this can be explained by two factors: (i) the greater number of potential breeding sites in Nilopolis and (ii) the LO was the only control method/device used against the target vector. The LO was not designed to be a sole control method/device for dengue vector control, but rather to be integrated with other control methods. Further field evaluations of the LO in other dengue endemic geographical locations and against other dengue vectors are planned. If such field tests prove the LO to be as efficacious as found in this study, then the LO could provide an inexpensive, simple, environmentally benign method to be integrated into the suppression of dengue vectors.

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