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 Nilopolis (~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...
mosquito that has adapted to utilizing man-made contain-
ers (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 and to 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 oviposi-
tion 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 Area 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) person-
nel. In addition, both municipalities are endemic with
dengue as reported by the Brazilian MOH. Two neighbour-
hoods, 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 Area 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 Nil-
opolis and Area 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 sam-
pling 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 popu-
lation 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 contain-
ers 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 collec-
tion. The adult mosquito collections were done by two
MOH persons using flashlights and battery-powered
hand-held aspirators (Hausher Machine Works, Tomy’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 Area
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 municipali-
ties 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
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 Area 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 Area 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 Area 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 houses.

### 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 (chi-squared test) as described in the text (c.f. Fig. 1a)

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Treatment</th>
<th>Mean % positive containers/house</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilopolis</td>
<td>Untreated</td>
<td>4.8</td>
<td>10.0</td>
<td></td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>LO treated</td>
<td>6.1</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area Branca</td>
<td>Untreated</td>
<td>13.2</td>
<td>17.6</td>
<td></td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>LO treated</td>
<td>10.3</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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)

<table>
<thead>
<tr>
<th>Municipality/treatment</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilopolis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>0.23</td>
<td>10.04</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>LO treated</td>
<td>1.03</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Areia Branca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>3.08</td>
<td>8.30</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>LO treated</td>
<td>1.97</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

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 than 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)

<table>
<thead>
<tr>
<th>Municipality/treatment</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilopolis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>0.53</td>
<td>2.67</td>
<td>P = 0.45</td>
</tr>
<tr>
<td>LO treated</td>
<td>0.70</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>Areia Branca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>0.36</td>
<td>6.83</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>LO treated</td>
<td>1.03</td>
<td>3.59</td>
<td></td>
</tr>
</tbody>
</table>

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.

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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 Aerea 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 Aerea 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 Aerea 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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References


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