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Aedes aegypti (Diptera: Culicidae): monitoring of populations to improve control strategies in Argentina

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Abstract To study the seasonal fluctuations of populations of *Aedes aegypti* (L.), to improve control strategies, or to monitor chemical control interventions, a lightweight, inexpensive ovitrap made of black plastic, pail-shaped, stackable, and provided with a wood tongue depressor was used. Field assays were performed in the northeast and northwest part of Argentina. In a 1-year trial performed in Tartagal (Salta), almost 100% of the ovitraps were highly positive, collecting a total of 1,000/2,000 eggs during March and the first part of April. A focal treatment in the corresponding neighborhood, performed at this time, immediately began to reduce positive ovitraps in spite of the high temperatures registered, rising again in November after winter season. Another field trial was performed in the whole urban area of Iguazú (Misiones). Mosquito populations were evaluated after three weekly ultra low volume (ULV) applications with an EC formulation of permethrin in water. The number of positive ovitraps diminished from 49% to 10% after the treatments. The last one performed in Wanda (Misiones) showed that positive ovitraps inside the dwellings aided in determining reinfestation rates after an intervention with a smoke-generating formulation containing beta-cypermethrin. The work performed in three different situations in urban areas at high risk of dengue can be considered a preliminary assay to establish the effective performance of simple ovitraps, allowing the Vector Control Service of the Argentinian Ministry of Health its use to improve surveillance and control strategies.

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Introduction

Aedes aegypti (L.) is the main dengue vector worldwide because of the close association with humans in tropical and subtropical highly inhabited areas. Around 1960, the vector was eradicated from Argentina; but since 1987, it was again detected in the northern region of the country with prevalence of DEN-1 and DEN-2 antibodies (Aviles et al. 1999; Carbajo et al. 2001; Boffi 2002). To date, there is still no effective drug or vaccine for treatment, control of the disease depends largely on the control of the vector. *A. aegypti* breed in natural and artificial containers in and around houses (Goma 1964). Unfortunately, campaigns to reduce vector populations have not been successful in urban areas (Matthews 1996). Thus, to reduce the incidence of the disease and control periodic outbreaks, the governments still depends on the conventional measures.

Because there is no dengue surveillance capability to detect an increased incidence of the vector to allow a timely response, most programs' reaction comes too late to alter the epidemic's course (WHO 1995a). Monitoring of *A.*

aegypti oviposition sites and performing preventive larviciding are the principal actions for *A. aegypti* control activities (WHO 1995b).

The implementation of any surveillance program for *A. aegypti* control depends largely on the sampling techniques and the dedication of time and money support. Vector surveillance gets information that can be of use in mosquito control, initiating control activities when mosquito larval or adult threshold populations are exceeded, to prevent a large outbreak, or recognizing changes in insecticide susceptibility to plan future control strategies (WHO 1995a).

Ovitraps, first used by Fay and Perry (1965), provide a sensitive and economic method for detecting the presence of *A. aegypti*. Ovitraps are in some ways superior to larval surveys (Fay and Eliason 1966), especially when density is low and larval indices are not effective (Jakob and Bevier 1969) and for detecting early infestations (Service 1976; Furlow and Young 1970). The ovitrap survey results in a specific, economic, and sensitive method for sampling populations. One inspector can cover three to five times more area per day with an oviposition survey than with a larval survey, and the cost is one-half to one-fourth the cost of a larval survey. Different devices used as ovitraps and oviposition preferences were evaluated both in laboratory and field assays (Thaggard and Eliason 1969; Kloter et al. 1983; Zeichner and Perich 1999; Perich et al. 2003; Lenhart et al. 2005) but no general recommendations were made. Although hay infusions have demonstrated to increase the probability of oviposition and resulted useful for day-to-day surveillance (Reiter and Nathan 1991), they were not so useful in weekly surveillances.

In this study, we assayed a light and economical plastic ovitrap with a medical tongue depressor as a method of monitoring population fluctuations to detect a peak of vector density and thus apply a control measure in the right moment or to evaluate the efficacy of different control measures in highly populated cities of northeast and northwest Argentina.

Materials and methods

Ovitraps Vessels made of semirigid black polypropylene plastic of 400 ml volume, 6.8 cm internal diameter at the bottom, 9.8 cm internal diameter at the top, 9.0 cm height, and 23.5 g weight were used. The clean ovitraps were each filled with 250 ml tap water, and a wooden tongue depressor or a cardboard paddle (18×150×1 mm) was fixed vertically inside the jar with a large paper clip. The ovitraps were inspected once a week and analyzed for *A. aegypti* eggs. The wooden paddles from the ovitraps were removed from the jars and, after counting the eggs, were discarded along with the water inside the jar. The jar was thoroughly cleaned with a paper towel in its inner surface, rinsed with clean water, subsequently refilled with tap water, and placed back in its respective location.

Study site The areas chosen for these studies were three subtropical urban areas in the north of Argentina. Iguazú is a large urban city with almost 30,000 inhabitants and located at 25°36′26.65″ S and 54°34′42.72″ W in the Misiones province. Wanda is a small village located at 25° 57′51.85″ S and 54°35′22.90″ W also in the Misiones province. Tartagal is located at 22°31′00.32″ S and 63°48′ 03.51″ W in the province of Salta.

Population monitoring Ovitraps were placed in shaded areas in the backyard of the house at a height of 1 m.

a) In Iguazú, 65 ovitraps were distributed using cluster sampling methodology (WHO 1995a), using a neighborhood as cluster. Then, simple random block design was used over this unit with two ovitraps per block throughout the whole city in urban and periurban areas (Gonzalez Gil et al. 1997). Monitoring was performed the week before the ULV treatment with permethrin and after each intervention. Fresh ovitraps were placed some time after the interventions. Three weekly ULV interventions were carried out by field workers between April and May.
b) In Wanda, an intervention with a smoke-generating formulation containing beta-cypermethrin was performed in one, well-limited area of the city. One ovitrap was left inside 25 of the 120 selected houses treated, usually under furniture, and the other one was placed in the backyard of the same house. The ovitraps were inspected before the treatment, cleaned, and water replaced after the treatment, and then inspected once a week up to 2 months.
c) In Tartagal, 30 ovitraps were located in the same way as previously described and inspected once a week starting on 11 January (calendar week 2) and ending on 27 December (calendar week 52).

ULV treatments Permethrin (cis/trans 45:55) EC formulation 10% (Imperator, Johnson Argentina S.A.) was used. The application was done at a concentration of 10 g a.i./ha in water with polyethylene glycol 6%. A ULV machine (Motan, Mobilstar, USA) carried on a truck driven at 10 km/h was used for spraying around each block (100×100 m) using 1 l/ha diluted insecticide and for spraying one cycle per week according to the standard WHO method (Pineda 1995).

The protocol for ULV control intervention used in Iguazú was according to the Vector Control Programme of Argentina.

Table 1 *A. aegypti* reinfestation rates in Wanda (Misiones, Argentina) after a treatment with a smoke-generating canister containing beta-cypermethrin inside the houses

Week post treatment	Percentage of positive ovitraps inside houses
Before treatment	20
1	0
2	0
3	5
5	16
6	10
7	28

Smoke-generating formulation treatment One canister (120 g) containing 5 g beta-cypermethrin (Bolate®, Chemotecnica S.A., Argentina) was placed in the middle of each dwelling with the outside doors and windows shut according to previous methodology (Masuh et al. 2003). The houses remained shut for 1 h and then were ventilated.

Results and discussion

A. aegypti is a domestic mosquito. About 87% of the Argentine population is concentrated in urban areas (INDEC 1991), thus favorable for the vector since they have more than 10 inhabitants per square kilometer or more than 2,000 inhabitants (Carbajo et al. 2001).

The distribution of ovitraps in urban and periurban areas of Tartagal, Iguazú, and Wanda cities was made according to the methodology established by the Health Ministry of Cuba for anti-*A. aegypti* campaigns. The number of ovitraps in each city was defined taking into account the density of houses of each area and the availability of manpower for such duties. Usually, ovitraps were placed one in each four blocks with dwellings but not less than 10% of the total blocks of the city.

No hay infusion was used in our study because traps were left in the field for 7 days. In Argentina, in urban areas, preliminary identification for the species of the collected eggs was made and only *A. aegypti* eggs were found in ovitraps. After each week of inspection, the ovitraps were thoroughly washed to ensure that no residual eggs adhered to the surface. On all occasions of treatments, no dead immature *Aedes* mosquitoes were noted in any of the ovitraps set up as a consequence of the chemicals used.

In Iguazú, population monitoring performed during the week before the ULV intervention resulted in 49% positive ovitraps. Because Iguazú City was at high risk of dengue vector transmission during the studies presented in this paper, the whole city was treated. After the first spatial ULV treatment with permethrin, the positive ovitraps were only 23%, falling to 9–12% in weeks 3 and 4. It can be seen that the percentage of positive ovitraps found in weeks 1 and 2 indicate the efficacy of the ULV treatment, but the values found in weeks 3 and 4 of the treatment had no significant difference. These results suggest that the ULV treatments significantly reduced the *A. aegypti* populations to a level which ovitraps could not determine. On the other hand, in areas far away from urban areas, the number of positive ovitraps was higher, probably because of a lower pressure of insecticide and of a higher availability of mosquito-breeding habitats.

In a previous study performed in Colonia Delicia (Misiones, Argentina), a new smoke-generating formulation containing beta-cypermethrin was assayed to control adult mosquito populations inside the houses (Masuh et al. 2003)

In the assay performed in the city of Wanda, one additional ovitrap was put inside of the dwelling to follow reinfestation after treatment with the smoke-generating canister. Before treatment, Breteau and house indices of 151% and 58%, respectively, were found and 20% positive ovitraps inside the houses. After the treatment of 120 dwellings with the smoke-generating formulation, only 0–5% positive ovitraps were found until week 3 after treatment; but from week 5 onwards, the percentage of positive ovitraps grew reaching 28% on week 7 (Table 1).

In Wanda, the high indices obtained before treatment showed the extension and density of mosquito infestation. After treatment with the smoke-generating formulation in which the smoke delivered could reach each hidden places inside the houses where mosquito rest, the positivity of the ovitraps became low, showing a low density of adult mosquitoes. In this way, reinfestation could be followed.

In the city of Tartagal (Fig. 1), the assay began in January (calendar week 2) with 64% of positive ovitraps

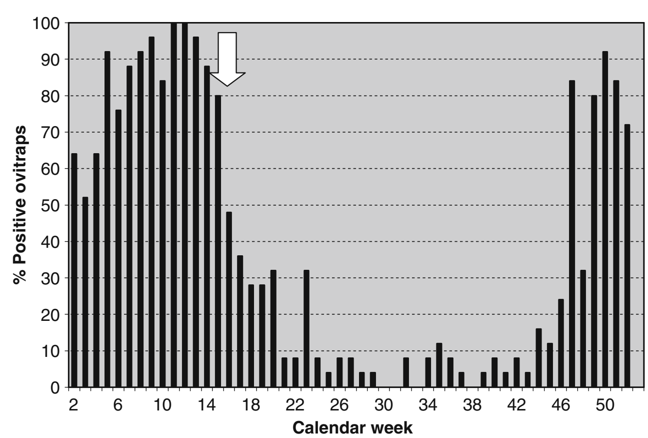

Fig. 1 Percentage of positive ovitraps in Tartagal City (Salta) during 1 year of population monitoring. The *arrow* indicates the time of larviciding in oviposition sites

growing to 100% in March with a total collection of 1,000–2,000 eggs. At the end of March, a focal treatment was performed in the whole city, and immediately, the number of positive ovitraps became fewer and remained at 0% in spite of the high temperatures registered until the winter months (July and August). During spring, the ovitraps became positive again reaching their highest values in December, during summer (week 50). A very similar distribution of positive ovitraps was seen during a preliminary assay. This fluctuation can be primarily associated with temperature variations from 5/10°C in winter to 35/40°C in summer months.

Artificial oviposition sites or ovitraps have been extensively used to detect the presence of *A. aegypti* (Service 1976). At the laboratory scale, we have previously demonstrated (Masuh et al. 2002) that these plastic, pail-shaped, and stackable vessels, which are very lightweight and easy to carry, were comparable in performance to a "CDC ovitrap" (Fay and Eliason 1966). In the different situations assayed, these ovitraps resulted in the effective monitoring of mosquito populations in urban areas at high risk of dengue in Argentina, even in areas difficult to access. Besides, the cost of these ovitraps is only US$ 0.30, which is very economical when compared with new designs of mosquito traps that have become available recently.

Conclusions

Controlling vector mosquitoes is an urgent public health problem in dengue-endemic countries. In dengue control, early warning systems to avoid outbreaks and a prompt answer for vector control are essential. The results shown indicate that the use of ovitraps, a nontraditional entomological evaluation technique, is practical for monitoring populations of *A. aegypti* to determine seasonal fluctuations and thus perform focal treatments in those places where more eggs were found. In addition, mosquito population monitoring would allow the evaluation of control treatments and improving control measures.

In the future, these ovitraps could be used to collect *A. aegypti* eggs to perform an extensive program to monitor mosquito resistance in different areas of our country.

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