Use of the pupal/demographic-survey technique to identify the epidemiologically important types of containers producing *Aedes aegypti* (L.) in a dengue-endemic area of Venezuela

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As dengue continues to emerge as a major public-health problem world-wide, efforts to control the dengue vector *Aedes aegypti* must become more effective and efficient. Results from larval and pupal surveys applied in Venezuela illustrate the uniqueness of the information gained from pupal surveys; information that is lost when traditional *Stegomyia* indices are calculated. As most *Ae. aegypti* pupae will emerge to become adults, controlling the containers that produce the most pupae could have the greatest impact on the adult population. Pupal-survey results in Venezuela showed that large (150- to 200-litre) water drums produce the greatest number of pupae throughout the year. In the rainy season, approximately 70% of all pupae are found in these drums or in tyres, buckets and tanks. Over 80% of pupae in the dry season are found in drums and tanks alone. By targeting only those domestic breeding containers that produce the greatest number of pupae, control efforts may be streamlined to have the greatest impact on reducing the local adult *Ae. aegypti* population.

Dengue is the most common and widespread arboviral disease of humans worldwide (Mairuhu *et al*., 1997). In the absence of a vaccine, control of the peridomestic vector mosquito, *Aedes aegypti* (L.), is the only effective preventive measure. Unfortunately, traditional *Stegomyia* indices do not provide a complete picture of the ‘key’ containers used by *Ae. aegypti* as breeding sites (i.e. those that are most important to the production of adult *Ae. aegypti* in a particular setting; Focks, 2003). As most (>80%) of the pupae of this species probably survive to become adults (Focks *et al*., 1993), pupal surveys would appear to be a reasonable method of identifying the types of container that are responsible for producing the bulk of a local population of adult mosquitoes. Such identification of the key container types should allow control efforts to be better focussed and more cost-effective.

The main objectives of the present study, which was based in the Venezuelan city of Trujillo, were to assess the consistency and practicality of using pupal/demographic surveys to identify the containers that are the most productive breeding sites for the local *Ae. aegypti* population.

**MATERIALS AND METHODS**

In a longitudinal study, four cross-sectional surveys were performed in the neighbourhood of Tres Esquinas, in the city of Trujillo, Venezuela. Trujillo City (9°22’ N, 70°26’ W) lies in north–western Venezuela at 800 m above sea level, has a population of approximately 53,000, is the capital of
Trujillo state, has a mean annual rainfall of 750 mm, and has air temperatures ranging from 16–37°C. There are normally two rainy seasons in this area — the first peaking in April/May and the second in November — and transmission of dengue, although perennial, tends to peak as each rainy season ends. The first cross-sectional survey, in April 2004, was timed to coincide with the beginning of the first period of dengue transmission in 2004. To obtain an understanding of the most productive container
habitats throughout the year, at times of varying rainfall and dengue-transmission risk, further surveys were conducted in May, June and October of the same year.

The neighbourhood of Tres Esquinas consists of approximately 1000 households. Each house was mapped using a handheld global-positioning system (Magellan Meridian® Gold; Thales Navigation, Santa
Clara, CA) and data were collected on all the water-holding (‘wet’) containers in the study area. A detailed survey that included the collection of data on container type, volume, location and use was conducted at household level. At the same time, the number of residents in each sampled household was recorded, so that the size of the human population of the neighbourhood could be estimated, and the results of the pupal survey could be expressed as the number of *Ae. aegypti* pupae/person — a statistic that can help to estimate the levels of reduction necessary to limit transmission.

In the first survey, significant efforts were made to survey every house in the community and 1031 houses were studied. In subsequent surveys, fewer households participated (774 in May, 737 in June, and 787 in October), as time constraints did not permit repeated visits to the houses where no-one was home when the survey team arrived. Each wet container detected was checked for the immature stages of mosquitoes. Every pupa seen was counted, collected (using pipettes, ladles and fine-mesh colanders), taken back to the laboratory, and allowed to develop into an adult, which was sexed and identified to species.

The data collection was carried out by 10 trained entomology technicians and overseen by a research entomologist and three other senior researchers. A data-entry technician entered all the field results into an Access (Microsoft) database.

**RESULTS**

In all four surveys, more pupae were encountered in large cylindrical water drums (with capacities of 150–200 litres) than in any other type of container (Fig. 1). This trend was most marked in the June survey, when 55% of all the pupae collected came from such drums. The percentage of all collected pupae coming from large plastic water tanks (found both at ground level and on the roofs of houses) was also much higher in the June survey (31.2%) than in the three other surveys. June coincided with the beginning of the first dry season, during which piped water is typically rationed and people begin to store large quantities of water, most commonly in drums and tanks. During wetter times of the year, when the local residents are less inclined to store large quantities of domestic water, other types of wet container increase in their relative abundance and become more important in terms of pupal production (Fig. 1). Nearly 70% of the pupae collected during a rainy season (i.e. in the April, May and October surveys) came from large buckets, tyres, tanks or drums.

For each category of container, the mean number of pupae/positive container varied over time (Fig. 2). Most notably, the mean number of pupae in each positive water tank more than doubled between May (28.2) and June (59.0), highlighting the importance of this type of water-storage container during dry conditions. Over the entire study period, only two containers (both 150- to 200-litre drums) were found with >500 pupae each — one with 538 pupae in the June survey and one with 589 in the October survey. These might both be considered atypical infestations, as the next most productive container, a water drum seen in the October survey, only contained 171 pupae.

The results of the pupal survey were compared with those of a traditional container survey (which measures the presence or absence of any stage of immature *Ae. aegypti* in all water-holding containers), taking into account the types of containers that were positive. Although the results of the traditional survey indicated that bottles were the container type most commonly infested with immature *Ae. aegypti*, the results of the pupal survey showed that the contribution of bottles to overall pupal production was negligible (Fig. 3).

The data collected in the cross-sectional surveys were used to calculate the mean number of *Ae. aegypti* pupae/person in each survey. Overall, these mean values
correlated only marginally well with the traditional Stegomyia indices, corresponding best with the Breteau index and poorest with the container index (see Table).

**DISCUSSION**

The present results indicate that the methodology of the pupal/demographic survey provides a useful framework for prioritising vector-control interventions, while highlighting the limitations of data collected in traditional Stegomyia surveys. In the present study, counting the total number of pupae in each container offered the maximum information for identifying the key types of container to target in campaigns for the control of *Ae. aegypti*, and so achieving maximum impact. In Trujillo, dengue transmission is year-round but peaks occur soon after periods of heavy rainfall. Since transmission is perennial, the year-round targeting of the more productive containers, with larviciding or source reduction, is recommended. By comparing the results of the pupal/demographic surveys with estimates of the corresponding traditional Stegomyia indices, it became apparent how the latter may greatly distort the true importance of certain container types in the production of *Ae. aegypti* pupae and, ultimately, adults.

Computing the numbers of pupae/person, instead of using the traditional indices that are based on all immature stages, gives a

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**TABLE.** The levels of correlation (shown as Pearson’s correlation coefficients ($r$)) between the pupae/person index (PPI), in each of the four cross-sectional surveys, and the corresponding values for the traditional Stegomyia indices

<table>
<thead>
<tr>
<th>Correlate with PPI</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI</td>
<td>1</td>
</tr>
<tr>
<td>Premises index*</td>
<td>0.87</td>
</tr>
<tr>
<td>Container index$^\dagger$</td>
<td>0.25</td>
</tr>
<tr>
<td>Breteau index$^\ddagger$</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Percentage of premises with immature stages of *Ae. aegypti*.

$^\dagger$Percentage of wet containers with immature stages of *Ae. aegypti*.

$^\ddagger$Number of containers with immature stages of *Ae. aegypti*/100 premises.
unique measure of infestation, which could be potentially useful in assessing the risk of dengue transmission in a particular setting at a given time.

In addressing the question of consistency of the pupal-survey results over time, it became apparent that water drums consistently produced more pupae than any other category of container. A year-round intervention targeted at such drums is therefore recommended. Other interventions could be tailored to the season. The recommended containers to target during the rainy season (in addition to drums) would be large buckets, tanks and tyres; these are all easily identifiable containers which, with the

![Pie chart (a)](image)

![Pie chart (b)](image)

![Pie chart (c)](image)

FIG. 3. The relative importance of each container type to the production of *Aedes aegypti* may be perceived differently according to which survey technique is used. Here, the data from the April 2004 survey are presented in three ways: as the percentages of the containers found to harbour any immature stage (a) or the pupae (b) of *Ae. aegypti*, and as the relative contribution of each container type to the local production of *Ae. aegypti* pupae (c). Although the counts of containers with any immature stages (a) indicated that bottles were by far the most important category of container, bottles contributed only slightly to pupal production (c).
drums, produced approximately 70% of the pupae during the rainy season. A dry-season intervention could be focused only on drums and water tanks — the two container types that together produced >80% of dry-season pupae. With the exception of tyres, nearly all of the most highly productive containers encountered in the present study were being used to store domestic water.

In terms of their practicality, the pupal/demographic surveys, while initially tedious, can potentially reduce the costs of intervention activities and make them quicker. Although it requires patience and precision to search for pupae and count every one found, interventions can be more efficiently targeted once a picture of the most productive containers emerges. An intervention targeted only at water drums (which, in the case of Trujillo only represented 3%–19% of all water-holding containers), for example, would require significantly less effort than an unfocused intervention that was designed to treat or eradicate all water-holding containers. The most productive containers need only be identified once, just before beginning an intervention.

It remains unknown if, by attacking only the more productive types of container, the overall pupal (and subsequently adult) population of *Ae. aegypti* will significantly decrease. Further research will help to answer this question, as well as to clarify the relationships between pupal production, the adult population and, ultimately, dengue transmission.

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REFERENCES

