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COMPARISON OF DIFFERENT USES OF ADULT TRAPS AND OVITRAPS FOR ASSESSING DENGUE VECTOR INFESTATION IN ENDEMIC AREAS

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ABSTRACT. This report presents a set of field experiments designed to assess different protocols for the use of ovitrap and MosquiTRAP, a promising new trap for dengue vector monitoring. Percentage of positive houses, calculated either by the use of 2 traps (outside + inside) or 1 trap (outside) per house, tended to increase with time of exposure, at similar rates. When the aim was either to obtain a qualitative index (*Aedes aegypti*–positive site) or to determine percentage of positive houses in a selected neighborhood, the use of 1 ovitrap per house with only 5 days of exposure at the peridomestic area was quite sensitive. Seven days of exposure was too long, as saturation is expected in some places. The number of eggs collected per site increased with the time of exposure in all sites. At the 3-day trap exposure, we were not able to discriminate neighborhoods in terms of egg productivity per house. At the 5-day trap exposure, a rank of 4 sites was achieved. There was no correlation between the number of adults caught in MosquiTRAPs and number of eggs collected in ovitraps, neither per neighborhood nor per house where both traps were simultaneously exposed for 7 days in the peridomestic area.

KEY WORDS *Aedes aegypti*, MosquiTRAP, ovitrap, vector monitoring, adult infestation

INTRODUCTION

One major problem for dengue vector control programs is to efficiently and effectively estimate and monitor adult mosquito frequency, distribution, and density. This information is fundamental for assessing disease risk transmission and for evaluating the impact of vector control strategies. Methods for estimating dengue vector infestation rely on surveys of immature breeding sites and/or the use of traps (Focks 2003). Surveys are the traditional, time-consuming approach, where a large fraction of houses are visited and inspected by trained health workers. The number of containers positive for immature stages is the basic variable measured. From this number, many indicators of infestation are generated (larvae/container, positive container/house, pupae/ha, to name a few). In comparison to surveys, traps may be less labor-intensive alternatives.

There is a myriad of traps available for collecting container-breeding mosquitoes (Focks 2003, Ritchie et al. 2003, Krockel et al. 2006). The ovitrap is considered to be very sensitive for the detection of *Aedes aegypti* L. in noninfested areas and for monitoring infestation levels in controlled areas. Besides being considered a surveillance tool, the ovitrap is also believed to help in controlling dengue vector (Cheng et al. 1982, Zeichner and Perich 1999). Conventionally, an ovitrap is placed inside the house and/or in a peridomestic environment where it often stays for 7 days. Infestation is then evaluated by the number of *Ae. aegypti* eggs present on the cardboard or wooden paddle put inside.

An alternative to the ovitrap is the sticky ovitrap, which holds the ovipositing gravid female mosquito (Service 1993). This sticky ovitrap has the potential advantage of providing a direct estimate of the number of adults instead of immatures in the ovitrap.

For vector control programs, the use of vector surveillance traps is an attractive alternative to the traditional labor-intensive household surveys. However, studies are required to standardize the use of these traps for surveillance purposes. This report presents a set of field experiments designed to assess different protocols and to develop basic guidelines on the use of ovitraps and adult traps in vector surveillance programs.

MATERIALS AND METHODS

Study area

Study sites were chosen at 4 Rio de Janeiro, Brazil, neighborhoods, representing distinct levels of *Ae. aegypti* house index (HI: percentage of houses infested with larvae and/or pupae; Connor and Monroe 1923), human density, vegetation coverage, and urbanization level (Table 1).

Urban and suburban sites have houses with 2–3 bedrooms, with peridomestic areas, and access

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Table 1. Data on vegetation, population density and house indices of various collection sites in Rio de Janeiro.

Neighborhood	Urban type	Vegetation coverage	Inhabitants/ha	House index
Amorim	Slum (*favela*)	Very low	901	11.2
Olaria	Urban	Moderate	169	3.8
Rocha	Urban	Moderate	72	8.8
Sulacap	Suburban	High	14	0.6

to sewage disposal and water supply. The suburban site has houses with larger peridomestic areas, covered by gardens and fruit trees. In contrast, the *favela* (slum) has poor access to sanitation, irregular garbage collection and water supply, leading residents to store water in large containers. The houses are small (rarely >1 room), frequently sharing at least 1 wall with a neighbor. The majority of houses lack a peridomestic area in the *favela*, and human commutation occurs through narrow alleys.

Traps

Ovitraps consisted of a black plastic container with 270 ml water and 30 ml of a 10% by weight aqueous hay infusion as an ovipositional attractant for *Ae. aegypti* females (Fay and Perry 1965, Fay and Eliason 1966). Hay infusions incubated for 7 days, were subsequently filtered through a 100-mesh screen, diluted with water, and transferred to ovitraps at the start of each experiment designed to assess the number eggs laid at different intervals (3, 5, or 7 days). The ovitrap had a wet wooden paddle measuring 12.5 × 2 cm attached to its inner border where the eggs were laid. The sticky adult trap—MosquiTRAP™ (patent pending, version 1.0; Ecovec Ltda., Belo Horizonte, Brazil) was physically similar to the ovitrap, but with the internal walls covered with a sticky card to hold the attracted females. The MosquiTRAPs were baited with 300 ml of tap water plus a synthetic ovipositional attractant, AtrAedes™ (patent pending, Ecovec Ltda., Belo Horizonte, Brazil) (Eiras et al. 2001), which was identified by volatiles from grass infusion of *Panicum maximum* Jacquard.

Once a trap was placed in a house and left for a few days, the outcome might be measured in 2 ways. One might declare the house positive if there was at least 1 egg of *Ae. aegypti* in the ovitrap or 1 adult in the sticky trap, or one might count the number of eggs or adults to try to infer population more quantitatively. At the neighborhood scale, especially the one that matters for control programs, the corresponding indicators of infestation are proportion of positive houses per neighborhood and mean number of eggs or adult females per house per neighborhood.

Experiments

In the 2 field experiments performed, experiment 1 used only ovitraps to 1) assess the number of collected eggs as affected by the number of days the trap is left in the field, and 2) compare infestation measured by placing 2 ovitraps per house (1 indoors and 1 outdoors) versus a single trap in the outside environment. Experiment 2 was performed to compare infestation indices measured by the traditional ovitrap and the MosquiTRAP.

Experiment 1: Ovitraps were installed in 2 randomly selected houses per block at each study site. At each house, 1 ovitrap was installed indoors and another outdoors (at the peridomestic area), totaling 80 traps per neighborhood (40 houses), except in the *favela*, where 40 traps were used (20 houses). Ovitraps were placed in secure, shaded places both indoors and outdoors and left for 3 days, when paddles were removed and replaced by new ones and a new mixture of water plus hay infusion was transferred to ovitraps. After the paddle replacement, ovitraps were left for 5 days. As before, the mixture of water plus hay infusion as well as paddles were replaced by new ones and left for 7 more days. All paddles collected were examined under a stereomicroscope and the number of eggs per paddle was recorded. Next, the eggs were left to hatch in order to determine species composition according to the keys to species of Consoli and Lourenço-de-Oliveira (1994).

Experiment 2: Using the same set of houses as in the 1st experiment, 1 traditional ovitrap and 1 sticky trap (MosquiTRAP) were placed in the peridomestic area. Both traps were examined after 7 days, when paddles and sticky card were removed and changed for new ones. The procedure was repeated for another 7 days. In the laboratory, paddles and sticky cards were inspected for eggs and adult females, respectively, using a stereomicroscope.

Statistical analysis

To test the effect of trap location (inside × outdoors) and number of days on the proportion of positive houses, we used chi-square tests. To test the effect of the number of days and trap location (inside and outdoors) on egg density, we used 2-way analysis of variance. To assess the effect of placing 1 trap versus 2 traps per house, we also calculated the percentage of false negatives that would result from the 1-trap protocol. The percentage of false negatives is an estimate of the probability of declaring a house

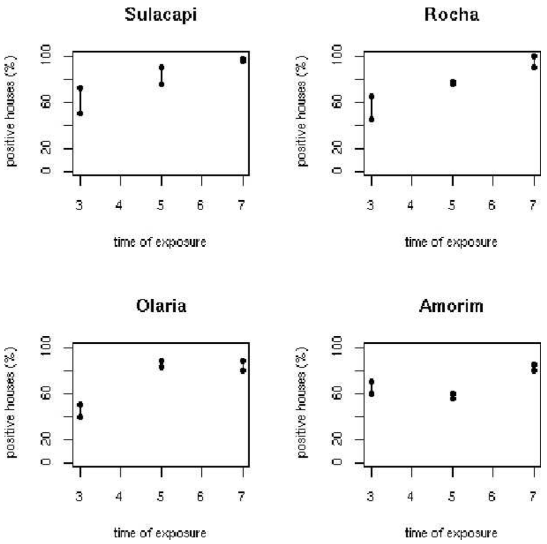

Fig. 1. Proportion of positive houses per study site, as a function of the ovitrap exposure time (days). Upper circles represent houses with positive ovitraps when using 2 traps per house (one placed inside and another outside); lower circles represent houses with positive ovitraps when using 1 trap per house (placed outside).

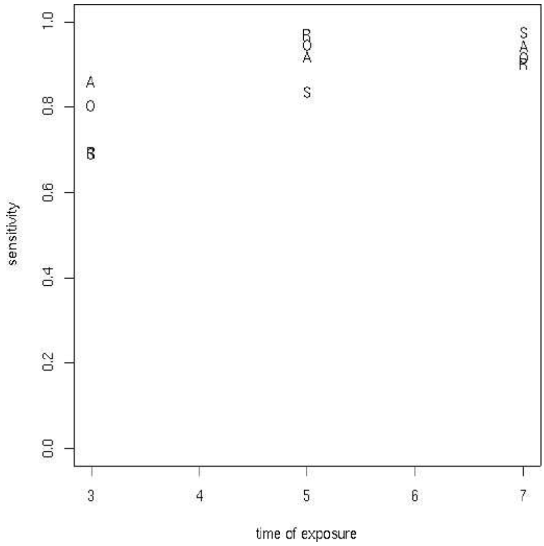

Fig. 2. Sensitivity of the outside ovitrap, compared with the outside and inside traps, as a function of the time of trap exposure (days). Letters correspond to the study sites: A, Amorim; O, Olaria; R, Rocha; S, Sulacap.

negative when the house would be positive by the 2-trap protocol (used here as the gold standard). Sensitivity of the 1-trap protocol (compared to the 2-trap protocol) is obtained by subtracting the proportion of false negatives from 1. The software R 2.5.1 (R. Foundation for Statistical Computing, Vienna, Austria; available from http://www.R-project.org) was used for the analyses.

RESULTS

Experiment 1: ovitraps

A total of 840 ovitraps were examined in experiment 1, and 61% of the ovitraps came out positive. *Aedes aegypti* was the dominant species in all areas, representing more than 98% of the eggs in the urban sites and the *favela*, and 90% in the suburban site. The remaining fraction was *Aedes albopictus* (Skuse). For the analyses below, we considered the outcome variable as the number of eggs per trap, without discriminating species.

Proportion of positive ovitraps per site: Data on the percentage of positive houses for *Ae. aegypti* eggs per area are presented in Fig. 1. The percentage of positive houses, calculated either by 2 traps or 1 trap per house, tended to increase with the time of exposure in all localities except for Amorim, where the proportion of positive houses was found independent of the exposure time ($\chi^2 = 4.06$, $P = 0.13$). With 2 traps, the percentage of positive houses varied from 50% to 75% at the 3-day exposure, 60% to 90% at the 5-day exposure, and from 80% to 100% at the 7-day exposure. The 1-trap protocol generated a lower percentage of positive houses, as expected. Discrepancies between the 2 indices tended to be greater at the 3-day exposure, especially in Sulacap (suburban) and Rocha (urban) (Sulacap: $\chi^2 = 20.3$, df = 1, $P < 0.001$; Rocha: $\chi^2 = 14.1$, df = 1, $P < 0.001$). At 5 and 7 days, differences were less than 5% in all areas (except Sulacap at 5 days, a difference of ca. 10% was statistically significant: $\chi^2 = 20$, df = 1, $P < 0.001$).

Using a single trap for 7 days, an average of 7% of infested houses would be erroneously declared as negative (i.e., 7% of the houses found positive with 2 traps would be regarded as negative if a single trap was used). This amount of false negatives varied between 0% and 17% among sites, with the exception of Sulacap where 50% of the infested houses would be missed by the 1-trap protocol. When the exposure time is reduced to 5 days, Olaria and Amorim showed values in the same range (0–17%). Sulacap and Rocha had high values (above 50%). With a 3-day trap exposure, all values are above 15% (Fig. 2).

Number of Ae. aegypti *eggs per site:* As expected, the number of eggs collected per site increased with the time of exposure (3 days: ca. 2,700 eggs in 114 positive traps; 5 days: ca. 9,800 eggs in 174 positive traps; 7 days: ca. 16,000 eggs in 223 traps). In Amorim (*favela*) and Sulacap (suburban), 72% of the collected eggs were found in the peridomestic trap. In the urban sites, Rocha and Olaria, this proportion was 64% and 66%, respectively. The proportion of eggs in the

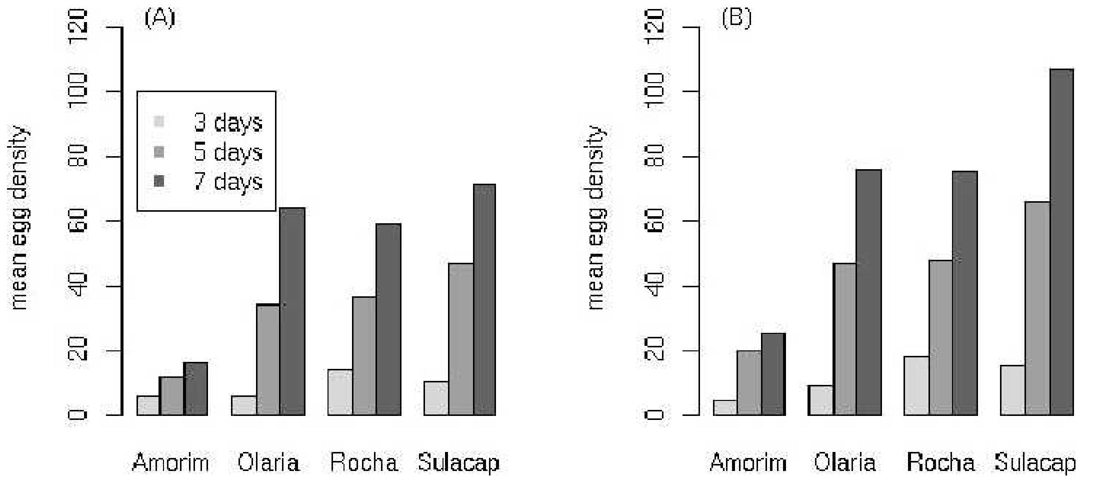

Fig. 3. Mean egg density per time of exposure in Amorim, Olaria, Rocha, and Sulacap. (A) Eggs collected using 2 traps per house (1 inside and 1 outdoors), and (B) only the outdoors ovitrap was considered.

outdoor traps did not change substantially as the time of exposure increased (Sulacap: from 70% to 74%; Rocha: 65% to 64%; Olaria: from 77% to 60%), except in Amorim where it jumped from 35% (with 3 days of exposure) to 77% to 86%, with 5 and 7 days (Fig. 3).

The time of exposure significantly contributed to mean egg density, while the location of the trap (inside or outside), did not affect it (Table 2). Among the sites, Amorim was the least infested, while the other 3 sites showed similar infestation levels.

Experiment 2: comparing ovitrap versus MosquiTRAP

The total number of females collected in MosquiTRAP per site varied from 5 in Amorim to 45 in Olaria. The number of eggs collected per ovitrap was 2 orders of magnitude greater than the number of females caught per MosquiTRAP, varying from ca. 1,000 in Amorim to 4,300 in Rocha. The number of adults per house collected in MosquiTRAP varied from 0 to 2 in Amorim, 0 to 3 in Rocha and Olaria, and 0 to 6 in Sulacap. The number of eggs per house varied widely among sites. We did not find a direct correlation between the number of adults caught in MosquiTRAP and number of eggs collected in ovitraps placed in the same houses when both traps were simultaneously exposed for 7 days in the peridomestic area.

The data in Fig. 4 show the 4 neighborhoods in terms of the total number of eggs (divided by 100) and total number of adults. Ovitraps at Amorim had the fewest eggs, followed by Olaria and Rocha. Ovitraps at Sulacap had the most eggs. MosquiTRAPs at Amorim also caught the fewest, but Olaria was found to have the greatest number of adult mosquitoes collected.

DISCUSSION

In the present report, we tested different protocols for estimating *Ae. aegypti* population using traps, as an alternative to the household larval surveys, that generate indices such as the HI traditionally used by the dengue vector control program in Rio de Janeiro. We found that, in terms of risk of dengue transmission, the surveyed sites in Rio de Janeiro would be ranked quite differently when considering the traditional HI versus the results from the use of traps. Briefly, the highest and lowest HI were reported in the *favela* (Amorim) and the suburban neighborhood (Sulacap), respectively (Table 1). Interestingly, egg production and the number of trapped ovipositing females were noticeably lower in the *favela* than in the mentioned suburb. An urban site (Olaria), which had the highest number of ovipositing females caught by the MosquiTRAP, contrasted with an HI of only 3.8. These results reinforce the notion that traditional indices have limitations in determining local adult

Table 2. Two-way ANOVA results for (log-transformed) mean egg density of *Aedes aegypti* in 4 study sites in Rio de Janeiro.

Site	Amorim		Olaria		Rocha		Sulacap	
Source	F	*P*	F	*P*	F	*P*	F	*P*
Exposure	10.068	0.0019	66.614	<0.001	54.209	<0.001	136.34	<0.001

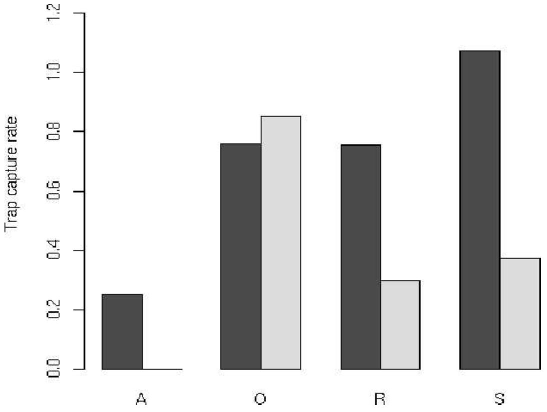

Fig. 4. Number of *Ae. aegypti* eggs per ovitrap/100 and adult female mosquitoes caught in sticky-trap MosquiTRAP operated simultaneously for 7 days in the peridomestic area of houses in 4 neighborhoods: Amorim (A), Olaria (O), Rocha (R), and Sulacap (S). Dark bars: total eggs divided by number of traps × 100, light bars: total adults captured divided by number of traps.

mosquito population and predicting dengue epidemics (Focks and Chadee 1997, Teixeira et al. 2002, Morato et al. 2005). The HI does not take into account larval-positive containers that will generate few or even no adult *Ae. aegypti* over time (Focks and Chadee 1997, Honório et al. 2006), and for many reasons some productive containers—the hot sites—are frequently overlooked by control workers (Focks 2003).

Using ovitraps, we found that in 1 wk all houses were eventually visited by the dengue vector in Rocha and Sulacap, and ca. 80% of houses in Amorim and Olaria. Trap-based indices are interesting because they measure number of houses at risk for dengue transmission, not the number or percentage of houses (HI) potentially producing mosquitoes (larval survey). The number of eggs laid in ovitraps and of stuck females in MosquiTRAPs showed that inhabitants living in the urban sites of Rocha and Olaria were exposed to *Ae. aegypti* more than in Sulacap, and much less in the *favela* (Amorim) (Figs. 3 and 4). Indeed, prevalence of reported dengue cases have been much higher in the above-mentioned urban sites than in the other neighborhoods surveyed (Municipal de Saúde do Rio de Janeiro, unpublished data). We therefore recommend the use of ovitraps and MosquiTRAP for detection and surveillance of *Ae. aegypti* in dengue control programs.

When the aim is to obtain a qualitative index (*Ae. aegypti*–positive site) or to determine the percentage of positive houses in such a neighborhood, the use of 1 ovitrap per house at the 5-day level of exposure at the peridomestic area is quite sensitive. According to our results, the percentage of positive houses, calculated either by 1 trap or 2 traps per house, tended to increase with the time of exposure at similar rates. The 7-day exposure is too long, as saturation is expected in some places. Indeed, using a sampling protocol with "only 1 trap per house" has a sensitivity of ca. 90% when compared to 2 traps per house, if traps are used for 5 or 7 days. This loss of sensitivity (compared to the 2-trap protocol) does not qualitatively affect the comparison between sites and exposure times. However, it does lose power when evaluating statistical differences among sites. One way to simply overcome this is to increase the number of houses sampled when using 1 trap at the peridomestic area. The choice of the peridomestic area to set the trap for attracting ovipositing *Ae. aegypti* is in accordance with the data obtained by Dibo et al. (2005) and Favaro et al. (2006). Maciel-de-Freitas et al. (2006) also recommended installing traps for collecting host-seeking females in the outdoors. In any case, entering houses to set and collect traps is obviously inconvenient for homeowners as well as for vector control workers.

One of the main uses of infestation indices is to rank neighborhoods according to infestation. Assuming that the 4 neighborhoods do have different levels of *Ae. aegypti* infestation, we found that the 3 days of exposure of ovitraps is too low to detect any difference between them. However, with the intermediate exposure of 5 days, the following rank is obtained: Sulacap is the most egg-productive site, Olaria and Rocha (the 2 urban sites) are tied in second place, while the *favela* (Amorim) is the least productive. Rocha would be defined as more productive for eggs than Olaria at the 7-day exposure, although egg counting in ovitraps does not correlate with the presumed infestation in terms of the number of *Ae. aegypti* females in such a place (Focks 2003). Saturation is expected with long exposure of ovitraps, i.e., eggs are found in essentially all ovitraps in some sites with the 7-day trap exposure. In fact, when using the MosquiTRAP, that potentially directly estimate the number of flying gravid *Ae. aegypti*, a clearer ranking was obtained, showing Olaria as the most infested area and confirming Amorim with the lowest adult infestation index.

MosquiTRAP has been shown to especially catch gravid females when compared with other physiological conditions of females caught in field studies (Favaro et al. 2006). It is an effective device for trapping *A. aegypti* when a larval survey would not detect the presence of this species (Gama et al. 2007). This sticky trap is more effective when placed outdoors (Favaro et al. 2006). Using varying numbers of MosquiTraps per block and their exposure times, more studies are needed to determine the best use of the sticky trap for assessing adult populations of *Ae. aegypti* in dengue endemic areas.

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