Infestation of *Aedes aegypti* estimated by oviposition traps in Brazil

Infestação por *Aedes aegypti* estimada por armadilha de oviposição em Salvador, Bahia

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Keywords
Aedes. Insect vectors. Oviposition. Ovitrap.

Abstract

Objective
To assess infestation levels of *Aedes aegypti* using the oviposition trap (ovitrap) method and to compare these results with data obtained with the use of indices traditionally applied in public programs aimed at fighting this vector.
Methods
Nine sentinel areas in Northeastern, Brazil, were assessed and infestation levels were measured for a nine-month period. Egg density and container indices were estimated and compared with previous results found using the house index and Breteau index.
Results
The results indicated that the area studied was infested with this vector during the entire study period and that the infestation was widespread in all areas. Different results were found with the different indices studied. There were areas in which the house index and the Breteau index were negative or close to zero, whereas the container index for the same area was 11% and the egg density index was 8.3%.
Conclusions
The container and egg density indices allow better assessment of infestation rates in a city than the conventionally used indices (house index and Breteau index). At lower operational costs and easier standardization, these indices can be applied as a measurement tool for assessing infestation rates during entomological surveillance in programs to fight *Aedes aegypti*.

Descritores
Aedes. *Insetos vetores. Oviposição. Ovitrampa.*

Resumo

Objetivo
Estimar os índices de infestação do Aedes aegypti*, utilizando ovitrampa com atrativo e comparar esse método com os tradicionalmente utilizados nos programas oficiais de combate ao vetor.*
Métodos
Foram analisadas nove áreas sentinelas de Salvador, Estado da Bahia, durante nove meses. Foram calculados os índices de densidade de ovos e positividade de ovitrampa, e levantamento dos índices de infestação predial e de Breteau para comparação.
Resultados
Observou-se que o município apresentou infestação pelo vetor durante todo o período de estudo em todas as áreas sentinelas. Os índices nem sempre apresentaram resultados

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de infestação semelhantes. Em algumas áreas os índices de infestação predial e de Breteau foram negativos ou próximos de zero, enquanto que o índice de positividade de ovitrampa apresentou valor de 11% e o índice de densidade de ovos 8,3%.

Conclusões

O índice de positividade de ovitrampa e o índice de densidade de ovos permitem avaliar melhor o quadro de infestação de uma cidade com custo operacional bastante reduzido e com maior facilidade de padronização do que os índices tradicionais (infestação predial e de Breteau). Recomenda-se, assim, sua utilização nas fases de levantamento de índices e de vigilância entomológica desenvolvidas pelo programa de combate ao Aedes aegypti.

INTRODUCTION

The complexity of the dynamics of dengue transmission has motivated researchers to carry out studies on the multiple factors related to the circulation and persistence of the virus in human communities.[5] Although some progress has been made in this field, much has still to be learned in order to guarantee more effective protection for populations, particularly in establishing the lower limits of infestation by the mosquitoes that transmit this virus, beneath which transmission to humans ceases to occur. There is still no scientific basis for establishing the minimum threshold of vectorial density that has to be reached in order to assure that the virus does not circulate.[5,6] In Salvador, Brazil, a recent study reported a high incidence of human infection in areas in which infestation indices were less than 3% as measured by conventional indicators.[7,9]

Given the need to evaluate the effectiveness of actions carried out in *Aedes aegypti* control programs and to establish sensitive risk prediction indicators, the development of accurate methods for defining vectorial density has become a very important issue. At the same time, it is vital that such methods be easily operated in order to streamline control programs. Conventional control programs have estimated vector density using the house index (HI) and the Breteau index (BI).[2]

Both HI and BI are the result of continuous, labor-intensive operations and depend on large contingents of trained human resources for quantification, thereby using significant portions of a control program's financial resources. In addition, standardization of the activities that have to be carried out is difficult and the quality of these activities, which require constant supervision, depends largely on the capability and responsibility of the ones in charge. Furthermore, the conventional methodology is uniformly applied with no consideration given to environmental or socioeconomic differences within each urban area. In addition to HI and BI, control programs also use systematic research at strategic points, as well as the installation of larva traps (larvitrap) during the entomological surveillance phase when HI and BI are below 3%.[2]

It is accepted that vectorial density indices routinely used in control programs of dengue mosquitoes are not sensitive enough to differentiate between situations of high and low risk of transmission. Therefore, these indices have been described in the literature as poor indicators.[4]

Other methodologies developed to increase early vector detection have proven to be of great use even in the entomologic surveillance of areas in which the vector is no longer found.[4] One of these is the trap model (ovitrap), which consists of a receptacle and pallet developed by Fay & Eliason.[3] Reiter & Gubler[6] further improved this original model by adding an infusion of diluted hay, which attracts the female *Aedes aegypti* to lay eggs. This technology has proven capable not only of detecting the presence of the mosquito, as did the conventional ovitrap that used tap water, but also of indirectly estimating the relative density of female mosquito population, by allowing the eggs deposited on the pallets that form an integral part of the trap to be counted. This means that the sensitivity of this method can be compared to that of HI and BI, as already described by Braga et al.[1] These authors, however, failed to take into consideration the comparison between the amount of eggs and larvae.

The objective of this study was therefore to assess the correlation between the amount of eggs deposited in these traps and larva density – as measured by the house and Breteau indices – in order to provide further data on the methodology of entomological surveillance and control of this vector.

METHODS

An aggregate study was carried out in the city of Salvador, capital of the state of Bahia in Northeastern Brazil. Salvador had a population of 2,443,107 inhabitants in the year 2000 and an overall mean house index for *Aedes aegypti* of 4.1% (range 2.2%-7.7%).*

From 30 sentinel areas in the municipality, nine were selected as study units comprising various conditions of environmental sanitation and families with different socio-economic levels[8] (Figure 1). The nine sentinel areas of the study were the following: *Armação, Barra, Calafate, Cobre, Lobato, Mangabeira, Médio Camurugipe, Paripe* and *Periperi*. In each of them, 10-13 areas of ovitrap coverage of approximately 10,000 m² were marked out, comprising a total of 107 units. The ovitraps were installed in the geographical center of these areas of coverage and were identified with individual numbering. *Calafate* was the unit with the smallest number of households (892) and *Lobato* had the largest one (2,287). A mean of 10-13 traps a week was installed in each sentinel area.

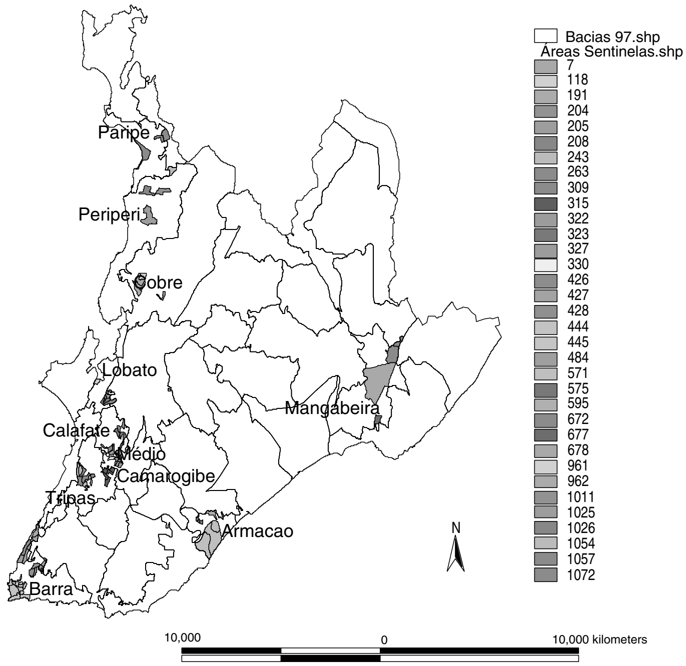

Figure 1 - Sentinel areas estimated by oviposition trap. Salvador, Bahia, Brazil.

Between August and December 2000, all buildings in all areas of coverage, a total of 11,639 buildings, were inspected monthly to collect larvae from hatching grounds. Between January and May 2001, 30% of these buildings were inspected, corresponding to a mean of 3,492 buildings per month.

The objective during each inspection was to identify and collect all mosquito larva found in all water-containing receptacles in and around the dwellings (water tanks, sinks, earthenware containers, tires, natural receptacles, wooden vessels, and disposable containers of various types). Collection was carried out using a lantern and special "larva-fishing" nets, except in the case of tires for which an aluminum scoop was used. The contents collected were transferred to a basin of clean water.[2] Larva collected were then aspirated using pipettes, placed in test tubes containing alcohol, labeled according to the place and date of collection, and transported to the entomological laboratory for identification of species.

Egg collection was carried out using ovitraps containing 10% hay infusion[6] in an attempt to make them more attractive for egg-laying. In case of ground-floor buildings, ovitraps were preferentially installed in the peri-domicile, at least one meter above ground level to avoid handling. In higher buildings they were placed on the first or second floors, always in shady, protected places inaccessible to children and domestic animals. The pallets were collected every five days to avoid handling and because the infusion loses its power of attraction over time. During this collection, the infusion was discarded and the ovitraps were cleaned, assuring the removal of any eggs deposited on the walls. Two days later, another trap was installed in the same place containing a new infusion and a new pallet.

At the entomology laboratory, the eggs that had adhered to the pallets were counted. They were maintained in laboratory conditions until embryonic development was complete. The pallets were immersed in dechlorinated water to allow egg eclosion. Identification of the species of larvae was carried out after the fourth development stage. As a result of the data obtained from the inspection of the buildings, an estimate was made of the infestation by *Aedes aegypti* using conventional indicators: HI (multiplying the number of positive buildings by 100 and dividing it by the total number of buildings inspected), and BI (multiplying the number of deposits with larvae by 100 buildings and dividing it by the number of buildings inspected).[2]

The container index (CI) is the ratio between the number of traps that were positive for *Aedes aegypti* and the number of traps installed. The egg density index (EDI) is estimated by dividing the total number of *Aedes aegypti* eggs found on the pallets by the number of positive ovitraps.[4] These indicators were calculated according to the month of occurrence and sentinel area, comprising a total of 69 measurements.

*In accordance with the report of 3rd cycle of the Program for *Aedes aegypti* Control in Salvador, 2000.

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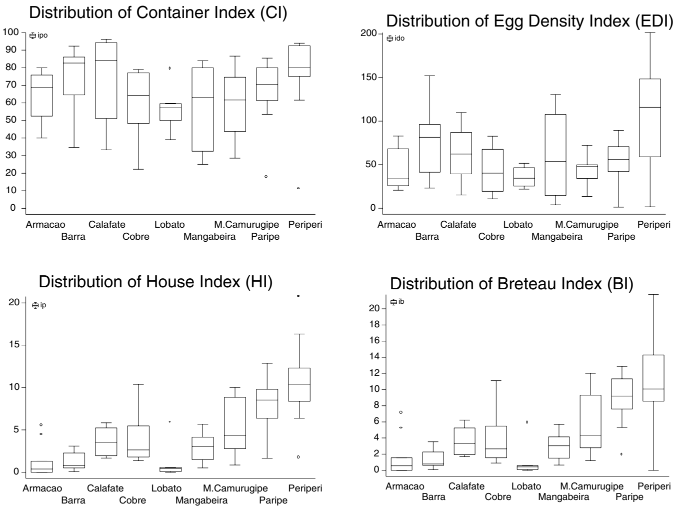

Figure 2 - Distribution of the container index (CI), egg density index (EDI), house index (HI) and Breteau index (BI) according to sentinel areas. Salvador, Brazil, September 2000 to May 2001.

The indices were presented as box-plots according to sentinel area and in historical series using a linear diagram with no adjustment model.The software programs used were Epi Info (v. 6.04), Excel and Stata.

RESULTS

Mean values of monthly CI observed varied from 57.1% (*Lobato*) to 75% (*Periperi*). The lowest mean index from the set of all the areas together occurred in September and the highest in January. Considering the whole study period, a mean CI of 65.1% was observed. The sentinel areas of *Barra*, *Calafate* and *Periperi* presented median CI higher than the general median (Figure 2).

Mean EDI varied between 35.6 (*Lobato*) and 106.2 eggs/ovitrap (*Periperi*), the lowest mean monthly index of the set of all areas occurred in the month of September and the highest in April. During every month studied, the mean EDI for all areas together was 60.0 eggs/ovitrap. The sentinel areas with EDI above the median value were *Barra, Calafate, Mangabeira, Paripe* and *Periperi* (Figure 2).

The HI presented mean monthly values of 1.2% (*Lobato*) to 10.7% (*Periperi*), the lowest mean monthly index was observed in October and the highest in May. The mean index for all areas was 4.5% and the areas of *Calafate, Mangabeira, Médio Camurugipe, Paripe* and *Periperi* presented values higher than the general median value (Figure 2).

The BI showed mean monthly values that varied between 1.2 (*Lobato*) and 11.7 positive deposits per 100 houses (*Periperi*). The lowest mean index occurred in October and the highest in March. The mean index for all areas during the entire study period was 4.8 positive deposits per 100 houses. The areas of *Calafate, Mangabeira, Médio Camurugipe, Paripe* and *Periperi* presented values above the median (Figure 2).

Based on the mean values of CI and EDI, the positive ovitraps indicated egg-laying and the presence of females in every area surveyed during every month of the study (Table). However, according to the house and Breteau indices, the areas of *Armação* and *Lobato* remained free from larvae during the months of February, March, April and October.

The four indices have not always indicated the same level of infestation by *Aedes aegypti*. For example, HI and BI registered zero in the months of October, March and April in *Armação*, while EDI indicated significant levels of infestation in this area, with an EDI of 33.8 and a CI of 52.5 eggs/ovitrap in October. Only in the sentinel areas of *Calafate* and *Paripe* all estimated indices were consistently above median values. Interestingly, the results revealed that both CI and EDI showed values above the median in the area with the best living conditions (*Barra*). A growth tendency was observed in all indices analyzed over the study period (Figure 3).

DISCUSSION

The infestation indices indicated the presence of *Aedes aegypti* during the study period over the entire geographic area studied. The areas of *Barra,*

Table - Distribution of means and confidence intervals of container and egg density indices according to house indices. Salvador, Brazil, August 2000 to May 2001.

House index	N	Container index		Egg density index	
(%)		Mean value (%)	95% CI	Mean value (eggs/ovitrap)	95% CI
0.001-0.49	10	58.0	46.6-69.4	33.9	23.5-44.4
0.50-1.00	8	60.5	44.4-76.6	45.2	27.5-63.0
1.01-2.99	18	64.4	55.1-73.8	55.6	38.0-73.2
3.00-6.99	16	68.8	22.5-82.1	74.68	47.2-102.2
7.00-20.8	17	68.7	56.4-81.3	73.0	48.8-97.2
Total	69				

Calafate and *Periperi* had the highest indices. The mean HI in Salvador in the year 2000 was estimated in 4.1% (range 2.2%-7.7%),* by the city health department, value very similar to the found in the nine sentinel areas done in the same time. The increase in levels of egg-laying (CI), which began in September, and in the larva index (HI), which began in October, reveals possible intensification in the number of females in the reproductive phase at that time of the year.

The ovitrap positivity observed in Salvador in the present study (65.1%) is higher than those values observed in a study carried out in October 1995,[1] in which the same trap was used, but without bait (25.1% and 14.1%, respectively). This difference may be attributed to the use of a diluted hay infusion in the ovitrap, whose power of attraction has been described by Reiter.[6]

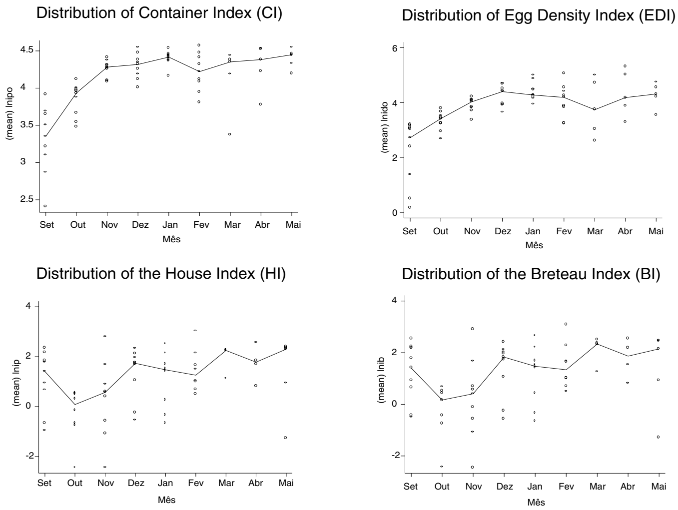

Figure 3 - Distribution of the container index (CI), egg density index (EDI), house index (HI) and Breteau index (BI) by month. Salvador, Brazil, September 2000 to May 2001.

Vector control actions have been carried out in the municipality. However, it is reasonable to assume from the positivity of the ovitraps and the number of eggs deposited throughout the study period that a constant replenishment of females was occurring. This would indicate that although these actions were insufficient to decrease the levels of infestation by *Aedes aegypti*, they were at least capable of restricting this increase to a controllable level.

A quantitative comparison of HI-BI and CI-EDI is not applicable, given that they measure different biological characteristics. The CI-EDI is estimated from the number of eggs detected in traps at a given time and describes the actual reproductive activity of the females in that environment. The HI-BI is based on larvae arising from the eclosion mechanism of eggs deposited over an indeterminate period of time (which could vary from days to months) in receptacles in the household environment.

However, the abundance of females, but not of larvae, in the sentinel area of *Barra* which was confirmed by high CI and low HI, may indicate fewer hatching grounds or the existence of a chemical barrier in this area since it is considered it unlikely that this finding is due to operational failures in the surveys carried out in this study. If this was the case, there would be a greater demand by the females for the traps that had been installed, increasing the egg density in the traps. It is also possible that the type of intervention carried out was effective in eliminating larvae but insufficient in its range. In areas such as *Periperi* and *Calafate*, high CI and HI indices may suggest a lower efficacy of control actions. The implementation of chemical control actions is complex, as sentinel areas have different geographical characteristics that would presumably favor different hatching grounds, and this should be considered when planning such actions. It is necessary to ensure that receptacles are covered and that toxicity levels of the chemical agent are controlled. The amount of water used by each household and the influence of natural factors should also be taken into consideration. It is important to take into account that receptacles exposed to rain, even when treated, may not respond as expected. The fact that there were areas classified as positive by EDI and negative according to HI and BI may indicate that the oviposition method is more accurate at estimating infestation. This may be useful in entomologic surveillance, even in areas traditionally considered as not infested. Consequently, the oviposition method as a measurement of infestation by *Aedes aegypti* seems to be more advantageous than larva methods. Its advantages rely on greater operational control with the potential of cost reduction, methodology standardization and fast identification of infestation. Larva methods instead, traditionally used in vector control programs, should be limited to sample studies since their use is more complex, whereas the oviposition method should be used for routine identification of vector infestation.

From the epidemiological point of view, the study results identified January and February as the months with the greatest likelihood of occurrence of cases due to the highest densities observed. This coincides with the months with the highest incidence of dengue, according to previous records in the city of Sal-

*In accordance with the report of 3rd cycle of the Program for *Aedes aegypti* Control in Salvador, 2000.

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vador and nationwide.[9] However, according to ovitrap positivity, it would seem that the city was constantly at an increased risk for dengue, as there were *Aedes aegypti* females feeding on humans.

Despite the variation in infestation levels according to the method used to identify the presence of *Aedes aegypti*, the fact that CI and EDI indicated infestation in areas in which HI and BI were negative may suggest the superiority of the oviposition method over the larva method. This finding confirms data already reported by Braga et al.[1]

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