Entomological Survey of Artificial Container Breeding Sites of Dengue Vectors in Batasan Hills, Quezon City

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ABSTRACT

Objective. Dengue fever remains a public health problem in the Philippines. Eliminating key container artificial breeding sites of mosquito vectors is a vital part of dengue control. The objective of this descriptive cross-sectional study was to conduct an entomological survey of artificial container breeding sites of *Aedes* mosquitoes in households of two puroks in Batasan Hills, Quezon City.

Methods. All potential artificial container breeding sites of dengue in each household were inspected for mosquito larvae. Water was sampled from all containers that had mosquito larvae and the larval species determined through microscopic examination. Using the World Health Organization list of recognized containers, each container was classified as a recognized container or an unrecognized container.

Results. The larval indices computed were: container index = 6.4%, household index = 23.9% and Breteau index = 29%. The proportion of containers positive for *A. aegypti* larvae was significantly higher for the unrecognized containers (9.9%) than that of the recognized containers (3.9%) (p=0.002).

Conclusion. The high household index and Breteau index indicate that the potential for dengue transmission is high in the study area. Unrecognized artificial containers contributed significantly to the number of *Aedes* breeding sites. "Search-and-destroy" campaigns in the community should be expanded to include these containers. Crafting specific vector control messages that address the problem of particular unrecognized containers as well as those of recognized containers with the highest proportion positive for *Aedes* larvae will also aid dengue control and prevention. Repeat surveys to monitor larval indices may be used to help ascertain the effectiveness of these messages in decreasing mosquito breeding sites.

Key Words: dengue, vectors, mosquito, Aedes, survey

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Introduction

Dengue fever, a mosquito-borne disease caused by the dengue virus, remains a public health problem in the Philippines. The disease was ranked tenth in the country's 5-year average morbidity data from 2000 to 2004.[1] In 2001, an estimated 528 disability-adjusted life years (DALYs) were lost to dengue throughout the world.[2] It has been demonstrated that the DALYs attributed to dengue nearly equaled the cumulative total DALYs for intestinal helminths, tuberculosis, malaria and the childhood disease cluster in Latin America and the Carribean.[2] The more severe forms of the disease are potentially fatal, as they are associated with bleeding complications and shock.[3,4]

The primary vector of dengue is *Aedes aegypti* while *Aedes albopictus* serves as a secondary vector.[2] *Aedes* mosquitoes lay their eggs in clean, stagnant water.[3,5] *A. aegypti* exhibits a preference for breeding in water-accumulating and water-holding artificial containers found within households.[5] *A. albopictus* was previously observed to exclusively breed in natural containers; however, recent studies have shown that *A. albopictus* breeds in artificial containers as well.[6,7]

The elimination of key artificial container breeding sites is regarded as one of best approaches for the prevention of dengue.[8] This strategy is part of "search-and-destroy" campaigns conducted for dengue prevention and control. Key containers differ from place to place.[9,10] By detecting previously unrecognized key containers, expansion of the coverage of "search-and-destroy" campaigns becomes possible. The reduced mosquito population resulting from the conduct of "search-and-destroy" campaigns will decrease vector–host contact, thus aiding dengue prevention.[9,11,12]

The World Health Organization (WHO) Guidelines on Dengue Surveillance and Mosquito Control lists recognized containers that have been established to be the usual breeding sites of dengue mosquito vectors (Table 1). Water tanks, drums, vases, pots, tin cans, pools, roof gutters, animal water containers, tires, discarded appliances, buckets, and ant traps are on this list.[6,12]

Table 1. List of WHO recognized artificial container breeding sites of dengue vectors[6]

Recognized containers according to WHO[6]		
Uncovered water tanks	Roof gutter	Ram barrels for collecting rainwater
Metal drums for water storage	Animal water container	Earthen water storage jars
Vases	Discarded tires	Concrete water storage tanks for bathrooms
Pots	Discarded appliances	Plastic containers
Discarded bottles and tin cans	Buckets	Water trays of refrigerators
Pools	Ant traps	Air conditioner trays
Saucers for ornamental potted plants		

Entomological surveys are primarily conducted to measure the relative presence of disease-carrying arthropod vectors.[13] The conduct of these surveys is recommended in countries endemic for dengue because survey results are useful for identifying possible causes of dengue outbreaks,[9] for identifying key containers,[5,8,9,14] and for identifying new breeding sites of vectors.[3,14] A survey is conducted by searching 100 or more households for possible breeding sites of vectors. In each household, all water-holding containers are examined, and water is sampled to detect the presence of vector larvae. Species identification is made after examination of larvae in the laboratory.[6,15]

Larval surveys utilize the Breteau index (BI), house index (HI) and container index (CI). BI refers to the number of larvae-positive artificial containers per 100 houses inspected. HI refers to the proportion of households with larvae-positive containers. The CI is the proportion of larvae-positive containers. The BI is regarded as the single most useful index for estimating *Aedes* density in an area because it is able to demonstrate a relationship between larvae-positive containers and the number of households. HI is used to determine the presence and distribution of *Aedes* in a locality and indicates that dengue virus may potentially spread in an area once a case becomes established. According to WHO guidelines, a HI >5% and/or a BI >20 in any area is a sign that the area is "dengue-sensitive", meaning that the risk of dengue transmission is high.[6] The HI and BI are frequently used to determine priority areas for the implementation of control measures.[6]

Barangay Batasan Hills in Quezon City, Philippines, is a highly urbanized and densely populated area where one of the highest incidence rates of dengue was observed in 2010.[16] In 2010, the *barangay* had a population of 150,764 and had the second largest population among all the *barangays* in Quezon City.[17] Residents in many parts of the area have to contend with poor living conditions, overcrowded households, high volumes of garbage, and substandard water supply systems that make it necessary to store water in tanks and barrels. In 2011, the water supply in Batasan Hills was available for only 8 to 12 hours a day.[18] These environmental conditions favor the breeding of dengue mosquito vectors that may prompt increased dengue transmission. In 2010, Batasan Hills was designated by the Philippine Department of Health (DOH) as a dengue "hotspot", a term applied to an area where clustering of dengue cases as well as an increasing number of cases have been observed for two consecutive weeks.[16] In the week from July 31 to August 6, 2011, the area was once more reported by the DOH as a dengue hotspot.[19]

The objective of this descriptive cross-sectional study was to conduct an entomological survey of artificial container breeding sites of *A. aegypti* and *A. albopictus* in households of two *puroks* (subdivisions of the *barangay*, the smallest administrative unit) in Batasan Hills, Quezon City.

Methods

Two *puroks*, Purok Ruivivar and Purok Baldago from Cluster VII, Barangay Batasan Hills, Quezon City were chosen as the sampling sites because the number of dengue cases recorded from these puroks for the first half of 2011 were among the highest for the Batasan Hills puroks, based on the review of records available at the barangay health center. Cluster VII is considered an urban poor community.

The study employed a one-stage stratified cluster systematic sampling design. The sampling unit was the household, defined as one unit of accommodation. The computed sample size was 162 households after taking into account an anticipated non-response rate of 10%. A total of 142 households were surveyed. The elementary unit comprised water-holding containers and artificial materials capable of accumulating water indoors and outdoors.

Household inspection

In each household, inspection for all potential artificial container breeding sites of dengue vectors was performed. Every room of each household was searched systematically for containers. The surrounding area of the household within a 5-meter distance from each wall of the house was also inspected for artificial containers.

All artificial containers were inspected for the presence of mosquito larvae through gross examination with the unaided eye. A flashlight was used for dark-colored containers in which mosquito larvae were more difficult to see. The number of containers inspected, type of containers, and the number of containers with at least one mosquito larva were recorded. Using the WHO list of recognized containers[6] (Table 1), each container inspected was classified as either a recognized container or an unrecognized container.

Sample collection

Water samples were collected only from containers that had mosquito larvae. For containers with a maximum volume of 500 ml, all the water contents of the containers were transferred to a labeled plastic bag. Dechlorinated water was added to the samples to prevent larvae from drying. For containers that held more than 500 ml of water, three representative samples were collected by the use of a standard entomological white and opaque dipper. The dipper was completely submerged in the container for less than 1 second to avoid disturbing the mosquito larvae and causing them to dive below the surface. All samples were transported to the laboratory for identification.

Laboratory identification

Larvae that were already at the fourth instar larval stage upon collection were immediately examined under a microscope to determine the species. Larvae collected at an earlier stage of development were reared in white plastic bowls to the fourth instar larval stage to enable species differentiation with the aid of a microscope. Upon reaching the fourth instar stage and prior to examination, larvae were killed by pouring hot water into the bowls containing them. Using a pipette, the larvae were aspirated from the water and mounted onto glass slides. By examining the characteristics of the comb scales found on the eighth abdominal segment of the mosquito larvae using the World Health Organization guide,[6] species differentiation was achieved. Containers that held at least one larva of either *A. aegypti* or *A. albopictus* were classified as positive. Containers that did not have any larvae or contained only non-*Aedes* larvae were classified as negative containers.

Figure 1 diagrams the process of data collection.

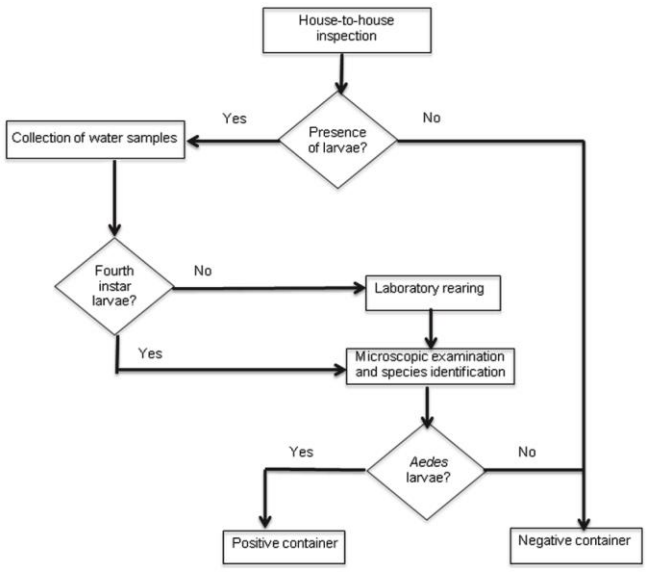

Figure 1. Process of data collection

Data analysis

Descriptive statistics, i.e., frequency and percentage distributions, were util ized to describe the containers identified in this survey. The BI, HI and CI for each type of container were computed using the following formulas:

$$CI = \frac{\text{number of positive containers}}{\text{total number of container breeding sites inspected}} \times 100$$

$$HI = \frac{\text{number of houses positive for Aedes larvae}}{\text{total number of households inspected}} \times 100$$

$$BI = \frac{\text{number of positive artificial containers}}{\text{total number of households inspected}} \times 100$$

The computed values were used as point estimates for generalizing the indexes to the target population. The 90% confidence interval estimates of the indexes were also computed using the following general formula for estimating the population proportion:

$$\hat{p} \pm Z \cdot \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

Meanwhile, the chi-square test of homogeneity was performed to compare the proportion of positive containers between the recognized and unrecognized containers. A 5% level of significance was used in making decisions for the hypotheses tested.

Microsoft Excel was used to encode the data and calculate descriptive statistics. OpenEpi v.2.3.1 (www.openpi.com) was employed in the computation of the interval estimates of the larval indices. Stata v.12 (StataCorp, College Station, Texas) was used to perform the chi-square test.

Ethics statement

The University of the Philippines College of Public Health Ethics Review Board reviewed and approved the research protocol before it was implemented. The investigators obtained informed consent from a member of each household prior to the conduct of the larval survey.

Results

Among 142 houses searched, 34 (23.9%) were found to have artificial containers that harbored *A. aegypti* larvae. Among 645 water-holding containers inspected, 41 (6.4%) were found to have *A. aegypti* larvae (Table 2). Four types of unrecognized artificial containers were found to be positive for *A. aegypti* larvae. *Culex* larvae were also detected in the study sites. *A. albopictus* and *Anopheles* larvae were not detected.

Table 2. Types of containers inspected and percentage positive for *Aedes* larvae

Type of container	Total number of containers inspected	Number of positive containers	Percentage of positive containers
Recognized containers			
Metal drum	164	11	6.7
Pots, vases, tin cans	16	2	12.5
Bucket	195	1	0.5
Animal water container	8	1	12.5
Total recognized containers	**383**	**15**	**3.9**
Unrecognized containers			
Dish organizer tray	83	22	26.5
Drum cover	15	2	13.3
Mugs in storage	3	1	33.3
Fountain	1	1	100
Basin	70	0	0
Bottle	22	0	0
Soap dish	11	0	0
Utensils holder	11	0	0
Dipper	10	0	0
Toothbrush holder	10	0	0
Water dispenser	7	0	0
Aquarium	4	0	0
Cups container	3	0	0
Bucket cover	3	0	0
Well	3	0	0
Container for baby bottles	2	0	0
Bowl	1	0	0
Shoe	1	0	0
Toilet brush holder	1	0	0
Total unrecognized containers	**262**	**26**	**9.9**
Total	**645**	**41**	**6.4**

Container index

With 41 out of 645 containers positive for *A. aegypti* larvae, the CI was 6.4%. Unrecognized positive containers were more numerous than recognized positive containers (Figures 2 and 3). Four types of recognized and 19 types of unrecognized artificial containers were inspected. Each of the four types of recognized containers had at least one container that was positive for *A. aegypti* larvae. On the other hand, at least one container was positive for larvae in 4 of 19 unrecognized container types (Table 2).

Drums were the recognized artificial container with the greatest proportion of positive individual containers. Of the unrecognized containers, 9.9% were positive for *A. aegypt*i larvae, compared with only 3.9% of recognized containers. About two-thirds of all positive containers were unrecognized containers (Table 3).

House index

With 34 out of 142 households having containers positive for *A. aegypti* larvae, the HI was 23.9%. Figure 4 shows the distribution of households in terms of the status of the containers examined (positive or negative), as well as the classification of the positive containers found.

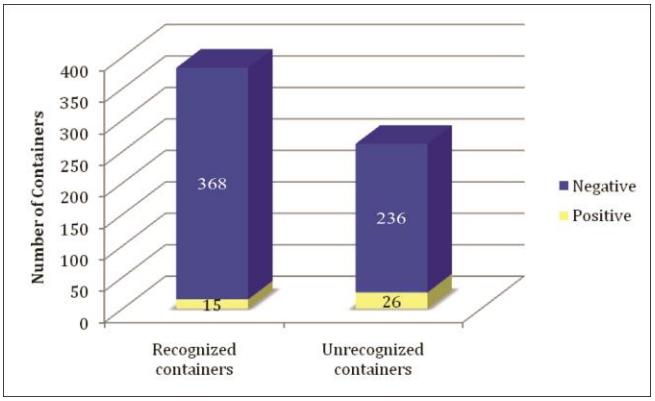

Figure 2. Distribution of containers according to type and positivity for *Aedes* larvae

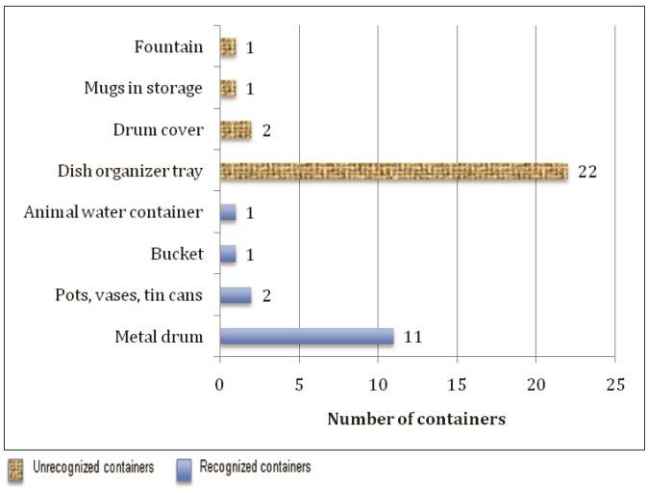

Figure 3. Frequency distribution of unrecognized and recognized containers positive for *Aedes* larvae

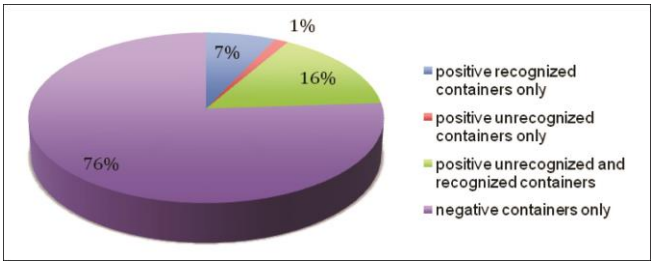

Figure 4. Distribution of households (N=142) in terms of positivity for *Aedes* larvae and type of containers identified within

Table 3. Number of positive containers classified as recognized and unrecognized

Container classification	Number of positive containers	Percentage of positive containers
Recognized	15	36.6
Unrecognized	26	63.4
Total	41	

Breteau index

Among 142 households, 41 positive artificial containers were found, yielding a BI of 29 artificial containers per 100 households.

Table 4 summarizes the three larval indices in the entomological survey.

Table 4. Summary of larval indices at 90% confidence level

Larval index	Point estimate	Interval estimate (90% CI)
Container index	6.4	4.78, 7.94
Household index	23.9	18.05, 29.83
Breteau index	29	22.62, 35.13

Discussion

The container types with the highest percentage of positive containers were dish organizer trays, drum covers, mugs in storage, plant pots, vases, tin cans, and animal water containers. Approximately half of all the containers surveyed were buckets and metal drums for storing water, both of which are WHO recognized containers. However, the proportion of positive containers among buckets and drums was only 0.5% and 6.7%, respectively. It is possible that water stored in these containers is used up and changed frequently owing to the irregular piped water supply in the area,[18] making the containers less favorable as mosquito breeding sites.

The proportion of positive containers that are not in the WHO list of recognized containers (i.e., unrecognized) is 2.5 times that of positive recognized containers. Of the recognized containers, only 3.9% were positive for *Aedes* mosquito larvae, in contrast with 9.9% of the unrecognized containers (Figure 2). Furthermore, the chi-square test shows that the proportion of positive containers is significantly higher for the unrecognized containers than for the recognized containers ($p=0.0002$). This demonstrates that the problem created by the presence of unrecognized containers that serve as mosquito breeding sites may be as important as that from recognized containers. Dish organizer trays topped the list of unrecognized containers that were positive for *Aedes* larvae (Figure 3). A possible reason for this finding is that residents may have lacked the awareness that these trays, though shallow, can accumulate and hold water long enough to serve as mosquito breeding sites. Another plausible reason is that dengue prevention campaigns in the area may have put greater focus on better-known mosquito breeding sites such as vases, water storage containers, old tires, and empty bottles and cans.[20] These proposed reasons may also explain the fact that a higher percentage of drum covers was positive for *Aedes* larvae compared to the drums themselves.

The term "key containers" has been applied to containers in households that consistently serve as the chief source of dengue mosquito vectors.[8] Identifying these key containers has facilitated the crafting of mosquito control messages that are specific for each type of container. This strategy has been identified as a best practice in the prevention and control of dengue in the Americas[8] and Australia.[10] Data from this study can therefore be used to help formulate specific messages that pertain to dish organizer trays, drum covers, mugs in storage, vases, tin cans, and animal water containers, all of which showed the greatest proportions positive for *Aedes* larvae.

A BI>20 is an indicator that a locality is dengue-sensitive, which means that the potential for transmission is high when a dengue case becomes established in the area[2,5,21] (Table 5). The BI of 28.8 obtained in this study describes the risk of transmission of dengue in the study area as intermediate between low and high.[2,21] The HI of 23.9% is evidence of a high risk of transmission (Table 5). It is important that the HI and BI be considered together because the HI provides information regarding the distribution of the positive containers within the households that are taken into account in determining the BI.

Table 5. Epidemiological interpretations of HI and BI[6,22]

Entomological Indices	High Risk of Transmission	Low Risk of Transmission
Breteau Index	>50	<5
House Index	>10%	<1%

Given the findings of this study, the conduct of campaigns that contain messages specifically addressing the problem of unrecognized and recognized key containers in the study area is recommended. Performing a repeat survey afterwards to check for an improvement in the larval indices may help determine the effectiveness of the campaigns.

Limitations

The study did not include artificial containers in less accessible areas of the households such as roofs and roof gutters in the survey. This may have resulted in an underestimation of the larval indices. Other unrecognized containers may also have been missed as a result.

Conclusion

The high HI and BI indicate that the potential for dengue transmission is high and that the potential for a dengue outbreak is high in the study area, indicating the need for strengthening dengue control and prevention measures. Unrecognized artificial containers were found to

contribute significantly to the number of positive containers. "Search-and-destroy" campaigns in the community should be expanded to include these unrecognized containers. Formulating specific vector control messages that address the problem of particular unrecognized containers, as well as those of recognized containers with the highest proportion positive for *Aedes* larvae will also aid dengue control and prevention. Repeat surveys to monitor larval indices may be used to help ascertain the success of these messages in decreasing mosquito breeding sites, as well as to generate useful information for refining future dengue control and prevention campaigns.

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