Distribution of dengue vectors in neighborhoods with different urbanization types of Manaus, state of Amazonas, Brazil

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Aedes aegypti *and* Ae. albopictus *are vectors of dengue viruses, which cause endemic disease in the city of Manaus, capital of the state of Amazonas, Brazil. More than 53 thousand cases have been registered in this city since the first epidemic in 1998. We evaluated the hypothesis that different ecological conditions result in different patterns of vector infestation in Manaus, by measuring the infestation level in four neighborhoods with different urbanization patterns, during the rainy (April), dry (August), and transitional (November) seasons.* Ae. aegypti *predominated throughout the study areas and sampling periods, representing 86% of all specimens collected in oviposition traps. High frequencies of houses positive for both species were observed in all studied sites, with* Ae. aegypti *present in more than 84% of the houses in all seasons.* Ae. albopictus, *on the other hand, showed more spatial and temporal variation in abundance. We found no association between infestation level and house traits. This study highlights the homogeneity of dengue vector distribution in Manaus.*

Key words: *Aedes aegypti* - *Aedes albopictus* - dengue - Manaus

Epidemics of dengue fever have been recorded in Brazil since 1981-1982 (Siqueira et al. 2005), beginning a few years after the recolonization of the country by *Aedes aegypti*, its primary vector. At present, this dengue vector species is widespread in Brazil and transmits three of the four dengue virus serotypes (Nogueira et al. 2002). In Brazil, *Ae. albopictus* is considered a potential vector of dengue, capable of transmitting these arboviruses and others under laboratory conditions (Schatzmayr 2000, Castro et al. 2004). This species is spreading throughout Brazil, causing concern (Santos 2003, Lourenço-de-Oliveira et al. 2004, Maciel-de-Freitas et al. 2006) because of its potential role as a vector of dengue and other arboviruses, as the yellow fever virus.

Manaus, capital of the state of Amazonas, currently with ca. 1,6 million inhabitants, is experiencing fast population growth (it doubled in the last 25 years). Amazonas was one of the last Brazilian states to be re-invaded by *Ae. aegypti*, in 1996 (Figueiredo et al. 2004), after its eradication in 1955 (Consoli & Lourenço-de-Oliveira 1994). This city's first modern dengue epidemic occurred in 1998, when 13,873 cases, caused by DEN1 and DEN2 sorotypes, were recorded. Since then, dengue fever cases have occurred routinely, totaling more than 53 thousand cases to date (Susam 2002, Funasa 2003). DEN3 serotype has been recorded in Manaus since 2003 **(**Araújo et al. 2003).

Dengue fever in Manaus has assumed a seasonal pattern, peaking during the rainy months from February to May (FMTAM 2005). Sporadic human cases have being recorded during the other months. A high level of *Ae. aegypti* infestation in Manaus, together with the presence of three dengue serotypes, raises concerns regarding the risk of new epidemics and emphasizes the need for efficient entomological surveillance strategies for mosquito control.

Mosquito control in Manaus relies on pesticide applications to reduce adult and larval populations, campaigns for larval habitat elimination, fast entomological surveys during critical periods of dengue transmission, and public education. Entomological surveys based on the presence of *Ae. aegypti* immatures in containers (Focks & Chadee 1997, Focks et al. 2000) continue to be the main surveillance tool in Manaus despite their inadequacy for dengue transmission risk assessment.

Ovitraps have been used for mosquito surveillance (Fay & Perry 1965), dispersal and spatial pattern detection (Honório et al. 2003, Costa-Ribeiro et al. 2006), assessment of the impact of control measures (Mogi et al. 1990), and for detecting key containers for adult mosquito production (Souza-Santos 1999, Braga et al. 2000). The ovitrap is a sensitive, fast and cheap tool to detect *Ae. aegypti* (Fay & Eliason 1966). Studies in Brazil devoted to the evaluation of ovitraps for surveillance have shown that ovitraps may detect *Ae. aegypti* in geographical areas where larval surveys do not (Braga et al. 2000).

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In Manaus, rapid urbanization associated with irregular sanitation and water supply, combined with local topology (low lands), and climate (year-round high temperature, humidity, and a long and intensive rainy season) create a perfect setting for dengue vector proliferation (Pinheiro & Tadei 2002). This work tests if neighborhoods with different urban conditions are associated with different levels of *Ae. aegypti* infestation in Manaus. Several authors have shown that the spatiotemporal distributions of *Ae. aegypti* and *Ae. albopictus* are associated with human activities, demographic density and climatic conditions (Kuno 1995, Alto & Juliano 2001).

MATERIALS AND METHODS

Study area - The city of Manaus is located in the Northern Region of Brazil at the confluence of the Negro and Amazonas rivers (3° 06' S; 60° 00' W). The municipality is surrounded by the Amazonian forest and covers an area of 377,4 km^2 divided into 56 neighborhoods. Manaus climate is "Ami" type (Köppen 1948), with the rainy season lasting from January and May and the dry season from June to September. A transitional period occurs between October and December.

Classification of Manaus neighborhoods - Manaus neighborhoods were classified into four types (Table I), using multivariate cluster analysis (Johnston 1978). Classification was based on socio-demographic-urban traits potentially associated with vector abundance (% houses among buildings; % buildings with indoor access to piped water; % area covered with vegetation). The first two indicators were obtained from the 2000 census (IBGE 2000). The third was estimated from the analysis of Landsat satellite images using the Spring program (available in http://www.cbers.inpe.br/download/manaus.jpg) (Camara et al. 1996). This analysis generated four classes of neighborhoods: class A corresponded to well developed residential neighborhoods, with a high proportion of houses, adequate urban services, and low vegetation coverage. Type B corresponded to well developed residential areas as well, mostly with houses with adequate access to urban services and relatively high vegetation coverage (compared to A). Type C included areas mostly covered with buildings, adequate access to water and few green areas. Type D corresponded to recently settled areas, with poor access to urban services, and few green areas.

Entomological survey - For the entomological survey, we chose one neighborhood of each type: Chapada (type A), Coroado (type B), Flores (type C), and Tancredo Neves (type D) (Fig. 1). Since neighborhoods were large and heterogeneous, we restricted the survey to an area of approximately 800 m^2 within each neighborhood. From street maps, we randomly sampled 50 pairs of coordinates censuring those less than 50 m apart. Householders were invited to participate in the study and, upon consent, were interviewed. We recorded information about the house (residential/commercial, system of waste collection and water supply, type of yard, house size) and the residents (number of inhabitants, schooling of the head of the family).

Three entomological surveys were carried out in 2004. One during the rainy season (April), one during the dry season (August), and one during the transition period (November-December/early rainy season, at the end of the dry season and beginning of rains) (Fig. 2). Two ovitraps were placed in the peridomiciliary environment of each house (where was easier to get permission from the residents), preferably in the garden or in a shady area (Dibo et al. 2005). An ovitrap consisted of a black plastic container filled with a mixture of water and hay infusion (270 ml:30 ml), and a wooden stick (15 cm × 2 cm) to collect eggs. Houses were revisited every seven days, for three weeks. At each visit, larvae in the ovitraps were collected as well as the wooden sticks, and hay infusion replaced. In the laboratory, the larvae were identified using a key modified from Consoli and Lourenço-de-Oliveira (1994) and counted. The sticks were maintained in a humid box for one week, air-dried and their eggs counted. The wooden sticks positive for eggs were immersed in water and emerged larvae were reared to the fourth instar, identified and counted.

Data analysis - For the analysis, we calculated two indices: the proportion of surveyed houses with positive traps (where "positive" means at least one egg or larva of *Ae. aegypti* and/or *Ae. albopictus* during the 3-week survey) and mean egg production per trap per week, calculated as the number of eggs collected per house

TABLE I

Classification of Manaus neighborhoods according to socio-demographic-urban variables associated with infestation risk

Neighborhood type	Type A	Type B	Type C	Type D
Proportion of houses	+++	+++	++	++
% houses among buildings	+++	++	+++	++
% buildings with indoor access to water	+++	+++	+++	+
% area covered with vegetation	+	+++	+	++

+ low; ++ intermediate; +++ high.
Type A: developed and residential neighborhoods, with a high proportion of houses, adequate urban services and low vegetation coverage; Type B: residential areas, mostly with houses with adequate access to urban services and relatively high vegetation coverage; Type C: areas mostly covered with buildings, adequate access to water and few green areas; Type D: recently settled areas, with poor access to urban services, and few green areas.

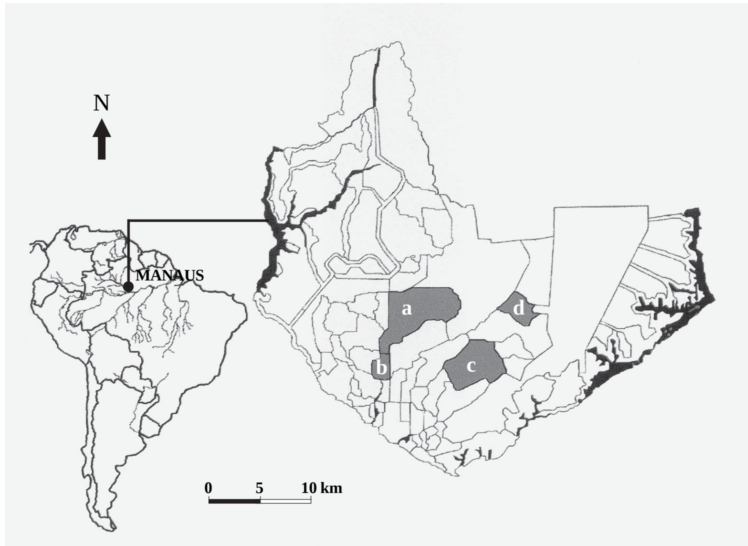

Fig. 1: map of Manaus showing the studied neighborhoods. a: Flores, b: Chapada, c: Coroado, d: Tancredo Neves.

divided by six (3 weeks × 2 traps per house). Eventually, some ovitraps were lost. Missing ovitraps were discounted from the original number.

Logistic regression analysis was performed to test for association between the presence/absence of *Ae. aegypti* (or *Ae. albopictus*) in each house (response variable) and site, season, and site vs season. Univariate and multivariate models (with and without interactions) were fitted to the data and the best model was chosen by comparing model deviances by Chi-square tests. For analysis of 'mean egg production', the same modeling procedure was applied, but using linear regression models, after log transformation of the response variable.

To test for possible associations between house traits and mosquito presence, we fit logistic regression models using presence/absence of *Ae. aegypti* (or *Ae. albopictus*) as response variable and house traits as covariates.

Pearson's and Spearman correlation tests were used to test for associations between *Ae. aegypti* or *Ae. albopictus* production (log-transformed). Only Pearson's tests are shown because of the similarity of the results. All analyses were performed using the software R 2.3.1 (R Development Core Team 2006).

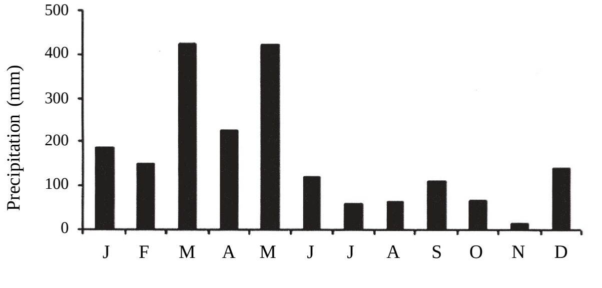

Fig. 2: monthly rainfall in the city of Manaus during 2004 (data provided by Instituto Nacional de Meteorologia).

RESULTS

Entomological survey - During the study 87,564 mosquito larvae were identified, 85.5% *Ae. aegypti* and 14.5% *Ae. albopictus*, the former species being more abundant in all sampling areas and seasons. A positive association was observed between *Ae. albopictus* and *Ae. aegypti* production, in all sites but Coroado (Chapada: Pearson's $r = 0.31$, df = 143, $p < 0.001$; Flores: $r = 0.36$, df = 148, $p < 0.001$; Tancredo: $r = 0.37$, df = 148, $p < 0.001$; Coroado: $r = -0.05$, df = 147, $p = 0.5$). Houses positive for *Ae. aegypti* were very common in all sites and seasons. The lowest prevalence values were observed in the dry season (August) when *Ae. aegypti* was found in 84 to 90% of the houses (Fig. 3), while the highest values were found in November (transition period), with 94 to 98% of the houses positive. When evaluating for effects of site, season, and site x season (using logistic regression), the model with 'season' as response variable was the best. Using the driest month as reference (August), the odds of finding a positive trap in November was 4.8 (95% IC:1.79-13, $p < 0.001$). April and August were not significantly different (Table II).

Ae. albopictus was less abundant in the rainy and dry seasons, increasing in the transition period (Fig. 4). This species showed variation among neighborhoods, being more abundant in Coroado, followed by Chapada. Tancredo Neves was the least infested. When fitting the logistic model, the effect of both season and site showed significance. The seasonal pattern of risk was very similar to that observed for *Ae. aegypti:* compared to the driest month (August), the odds of finding a positive trap in November was 4.6 (95% IC:2.7-7.89; $p < 0.001$) (Table II). No significant difference again, between August and April.

Egg production of *Ae. aegypti* was homogeneous throughout the four sites, with a mean of 42 eggs per trap (max = 376). April (the rainy month) produced the least number of eggs per trap (mean = 33; std = 48) while

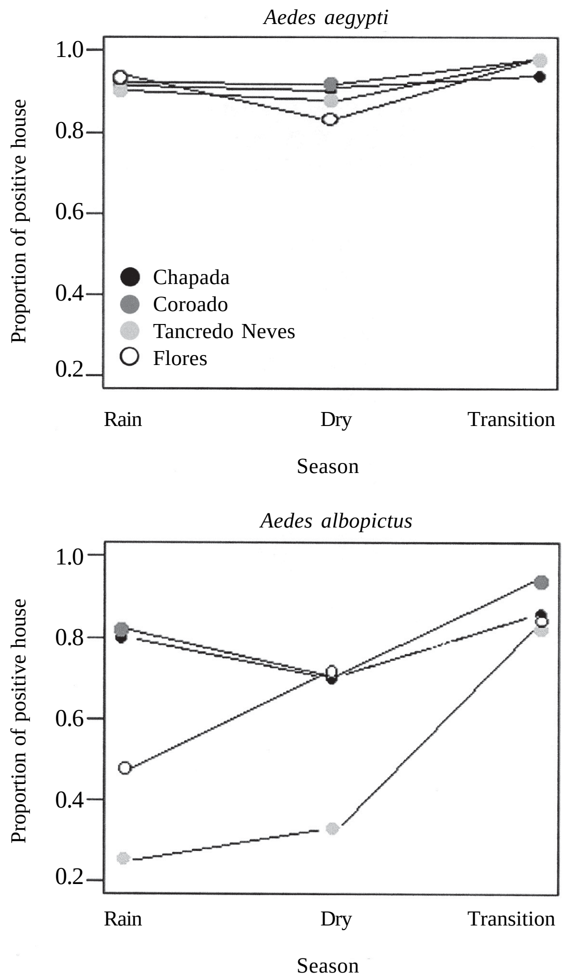

Fig. 3: proportion of surveyed houses positive for *Ae. aegypti* and *Ae. albopictus* in four neighborhoods of Manaus (Chapada, Coroado, Flores, Tancredo Neves), during the rainy, dry, and transition seasons of 2004.

the other two seasons showed similar values [mean = 47; std = 46 (Nov), std = 55 (Aug)]. Among the three models tested for *Ae. aegypti* egg production, the best one had only the term 'season' (Table III). November was again, the most productive month.

We found no association between any house trait, obtained during the survey, and *A. aegypti* infestation: sewage system (χ^2 = 0.001, df = 1, p = 0.975), water pipe (χ^2 = 2.05, df = 1, p = 0.15), type of courtyard (χ^2 = 0.042, df = 2, p = 0.97), schooling of the family leader (χ^2= 1.92, df = 3, p = 0.59) (Table IV). When comparing the median number of residents and rooms between positive and negative houses, we found no significant difference (residents: Wilcoxon W = 8778.5, p = 0.22; rooms: Wilcoxon W = 9171.5, p = 32). Without significant effects, no further modeling was attempt. Regarding *Ae. albopictus*, univariate analysis show significant effects of sewage and type of yard, but these effects vanished when neighborhood was taken into account (results not shown).

DISCUSSION

Manaus neighborhoods were classified into four types according to social and environmental characteristics potentially associated with *Ae. aegypti* infestation, nevertheless there were no significant differences among them in abundances of *Ae. aegypti* immatures. *Ae. aegypti* showed a wide and apparently uniform distribution across the city of Manaus, with high numbers in all surveyed areas during the three periods. We expected a higher abundance of *Ae. aegypti* in Tancredo Neves due to the large number of water reservoirs used for water storage. Although infestation was very high in Tancredo Neves, similar levels were also found in the other sites. At the local scale, we found no associations between house characteristics (such as sewage system, water pipes, kind of yard, schooling, number of residents, number of rooms), and the abundance of *Ae. aegypti*. Pinheiro and Tadei (2002), on the other hand, found

TABLE II

Results from the logistic model for the presence of *Aedes aegypti* or *Ae. albopictus* in traps, considering as potential predictors: site, season, and site vs season

	Ae. aegypti			
Variable	OR	95%OR	z-value	p-value
Season August	1			
Season April	1.52	0.76 - 3.03	1.202	0.229
Season November	4.82	1.79 - 13.0	3.108	0.001
	Ae. albopictus			
Variable	OR	95%OR	z-value	p-value
Season August	1			
Season April	0.80	0.51 - 1.23	2.684	0.31
Season November	4.62	2.70 - 7.89	5.59	< 0.001
Site Tancredo	1			
Site Chapada	6.11	3.49 - 10.69	6.34	< 0.001
Site Coroado	6.97	3.95 - 12.30	6.71	< 0.001
Site Flores	2.72	1.65 - 4.49	3.91	< 0.001

OR: odds ratio.

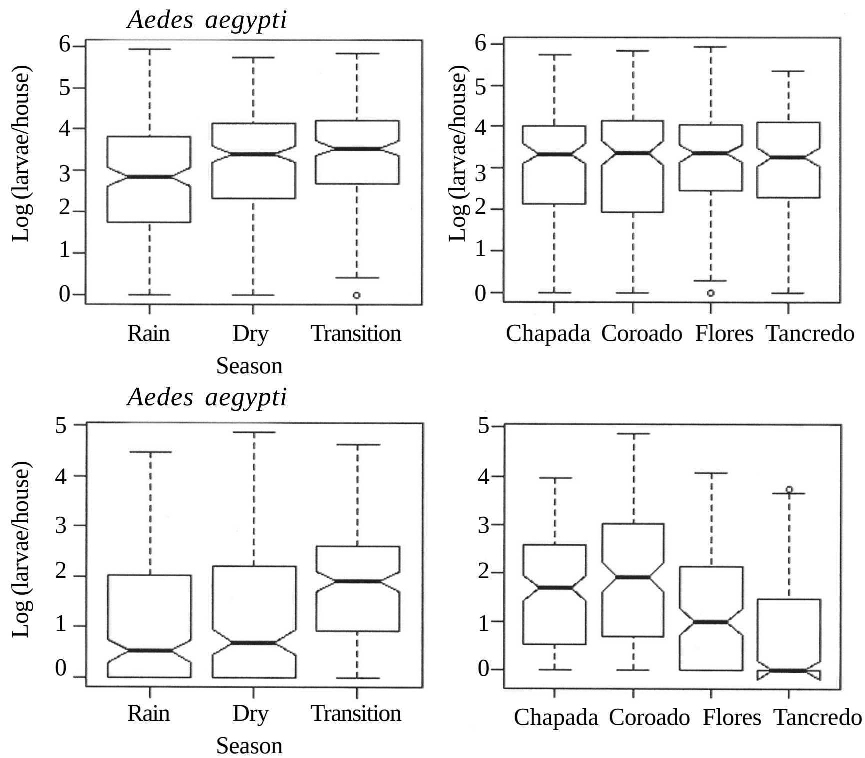

Fig. 4: boxplot of week mean number of *Ae. aegypti* and *Ae. albopictus* larvae captured per house (log scale) stratified per season and per neighborhood in Manaus, 2004 (Notches indicate median 95% confidence intervals).

TABLE III

Results from the linear model for the mean egg production of *Aedes aegypti* or *Aedes albopictus* per trap, considering as potential predictors: site, season, and site vs season

	Ae. aegypti (R^2 = 0.06)			
Variable	Estimate	95%CI	t-value	p-value
Season April	-0.392	[-.66,-.11]	-2.778	0.005
Season November	0.252	[-.02,.53]	1.78	0.07
	Ae. albopictus (R^2 = 0.21)			
Variable	Estimate	95%CI	t-value	p-value
Season April	0.00	[-.58,.33]	0.02	0.98
Season November	1.35	[-.16,.75]	5.94	< 0.001
Site Chapada	1.23	[.78,1.69]	5.31	< 0.001
Site Coroado	1.29	[.84,1.74]	5.68	< 0.001
Site Flores	0.98	[.53,1.42]	4.29	< 0.001
Chapada × Nov	-1.05	[-1.69,-0.41]	-3.2	0.0012
Coroado × Nov	-0.68	[-1.32,-0.05]	-2.13	0.033
Flores × Nov	-1.23	[-1.87,-0.6]	-3.83	0.0001

higher infestation of *Ae. aegypti* in Manaus in neighborhoods with poorer social conditions and water reservoirs, owing to the paucity of piped water. These authors assessing all water containers showing potential for harbouring and breeding of *Ae. aegypti* in two different neighborhoods of Manaus, but the method to evaluate the social and water supply conditions was not explained. Maciel-de-Freitas et al (2007), evaluating productivity of containers in Rio de Janeiro, Brazil, found higher mean numbers of pupae per house in well infrastructured district than in a slum. All of these results must be considered with attention because there are many factors influencing the *Ae. aegypti* infestation indexes, and every place has its particular characteristics.

A homogeneous distribution of *Ae. aegypti* was also observed by Getis et al. (2003), in the Amazonian city

TABLE IV

Characteristics of residents and houses in the neighborhoods surveyed

Sampled neighborhoods	Chapada	Coroado	Flores	Tancredo Neves
% with adequate sewage service	62	50	30	36
Piped water	84	74	68	68
Yard (with trees; cement; no)	18; 50; 26	48; 46; 4	50; 44; 0	84; 12; 0
No. rooms (median, min, max)	5 (2-22)	5 (1-10)	6 (2-16)	4.5 (2-10)
Residents/room (mean)	0.84	1	0.88	1.36
Schooling chief (median)	High school	High school	High school	Basic
% graduated	18	8	12	0

of Iquitos, Peru. This result may be explained by the relatively high availability of artificial containers throughout that city, high rainfall, and temperature. The climate in the Amazon region presents rather subtle variations. Although precipitation is lower during the dry season, it still is enough to maintain natural and artificial larval habitats. This pattern is distinct from other regions in Brazil, where greater numbers of *Ae. aegypti* are observed during the summer, when temperature and rainfall are high (Souza-Santos 1999, Glasser & Gomes 2002, Honório et al. 2006).

We observed a slightly lower abundance of both species during the dry season compared to the rainy season, as found by Pinheiro and Tadei (2002) in Manaus and Mahadev et al. (2004) in India. These authors indicated April (the month with highest precipitation) as the month with highest abundance of *Ae. aegypti* and *Ae. albopictus*, but they did not assess the transitional period (November). This was the period when we observed a significant increase in the abundance of both species. In the transitional period, low precipitation is sufficient to maintain habitats and hatch eggs accumulated during the dry season. Although large number of dengue cases in Manaus has been observed in the rainy season, a considerable number of dengue cases has been reported in the transitional or dry seasons, which demonstrates endemic dengue transmission occurring the entire year (Siqueira et al. 2005, Pinheiro et al. 2005). The strong control measures against both immature and adult mosquito forms, applied in the city of Manaus by the Fundação Nacional de Saúde (Funasa), during the rainy season, could diminish the mosquito populations. Detailed studies about that fact must be done.

Ae. albopictus was found in all sites, although more abundantly in the "greener" neighborhoods. This species has colonized different areas in Manaus, including recent human settlements and totally urbanized areas (Fé et al. 2003, Sá 2004). The presence of houses with open yards, next to forest fragments, enhances risk for *Ae. albopictus* (Passos et al. 2003, Lourenço-de-Oliveira et al. 2004, Mahadev et al. 2004, Maciel-de-Freitas et al. 2006), as in the Coroado neighborhood.

Although control strategies tend to be promoted during the rainy season, we suggest that control measures should consider the transitional period as a potential time for implementation.

In conclusion, our results point to the importance of the transition season as a period for vector surveillance and control. Our results also point to the potential benefit of using sentinel sites for vector surveillance in Manaus. Probably a sample of randomly chosen neighborhoods could be used as sentinel areas year-round, since all neighborhoods show similar infestation levels in any season. A survey with more neighborhoods should be done in the future to confirm these results.

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