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Communication/Comunicação

Breeding sites of *Aedes aegypti* in metropolitan vacant lots in Greater Vitória, State of Espírito Santo, Brazil

Criadouros de *Aedes aegypti* em terrenos baldios na região metropolitana da Grande Vitória, Estado do Espírito Santo

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ABSTRACT

Introduction: This study aimed to evaluate the presence of *Aedes aegypti* in breeding sites located in vacant lots (VLs) and determine the effectiveness of VL cleaning to reduce insect foci. **Methods:** Two types of VLs were sampled, the experimental VL, which was cleaned monthly, and the control VL, which was not cleaned. **Results:** Monthly cleaning of VLs reduced the abundance of immature forms of *A. aegypti*. **Conclusions:** Strategies for combating this vector should include regular cleaning of VLs and educating the public regarding the risks of discarding waste in inappropriate areas.

Keywords: *Aedes aegypti*. Breeding sites. Vacant lots.

RESUMO

Introdução: Os objetivos deste estudo foram avaliar a ocorrência de *Aedes aegypti* em diferentes tipos de criadouros em terrenos baldios (TBs) e os efeitos da limpeza mensal do terreno na redução dos focos do inseto. **Métodos:** Dois tipos de TBs foram amostrados, TB experimental submetido à limpeza mensal e TB controle, sem limpeza prévia. **Resultados:** A limpeza mensal dos TBs reduziu significativamente a abundância de formas imaturas do inseto. **Conclusões:** Estratégias de combate ao vetor devem incluir a limpeza de TBs e a conscientização da população sobre o risco que representa o descarte de lixo em local impróprio.

Palavras-chaves: *Aedes aegypti*. Criadouros. Terrenos baldios.

Measures taken for dengue prevention have focused on controlling the incidence of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in inhabited areas by searching for breeding sites in domestic/peridomestic areas, in addition to other likely places, such as cemeteries, scrap heaps and auto repair shops[1,2]. However, minimal attention has been given to vacant lots (VLs) adjacent to human habitations, where trash is commonly discarded by a considerable percentage of the population[3,4].

It is known that dengue epidemics escalate in periods of heavy rainfall and that several containers that could accumulate rainwater are found in VLs[5]. This makes VLs a significant source of *A. aegypti* breeding sites[6]. The present study aimed to: I) investigate the presence of immature forms of this insect in different types of containers; II) evaluate the effect of monthly cleaning on the reduction of insect foci and III) examine the influence of rainfall on the abundance of the insect. Overall, this study aimed to elucidate the role of VLs in the maintenance of breeding sites of this vector.

The study was conducted in VLs identified within the metropolitan region of Greater Vitória, State of Espírito Santo, Brazil. The region comprises of an area of 2,331km^2, of which 319km^2 constitute urban areas. Among the 393,799 permanent urban residents, 10,460 (2.7%) dispose of domestic trash in VLs or on the streets[7].

The VLs were chosen from neighborhoods with similar geographic and socioeconomic characteristics, each situated in peripheral areas of the city (**Figure 1**). The VLs were defined as vacant areas comprising approximately 900m^2, without any buildings or houses, and where the general population frequently deposited domestic trash.

The sampling procedure included identifying breeding sites present in the VLs, collecting Culicidae immature forms, and a final cleaning of the VLs; i.e., removing all artifacts capable of accumulating water.

Two categories of VLs were defined in this study: I) experimental vacant lot (EVL), defined as a fixed VL that was submitted to an initial cleaning in which all artifacts capable of accumulating rainwater were removed 30 days prior to sampling. Subsequently, monthly sampling (including cleaning) was performed throughout the course of one year to determine the number of breeding sites present and the effectiveness of periodic cleaning in reducing this number; and II) control vacant lot (CVL), defined as an area similar to an EVL, but which was not cleaned prior to sampling. From the scientific point of view, the CVL should remain untouched after the identification of breeding sites and the collection of Culicidae immature forms. However, due to ethical concerns, all artifacts capable of accumulating rainwater were removed following sampling. As a result of this post-sampling cleaning, the CVL was unsuitable for the following month's sampling and a new CVL was chosen for each month's sampling. The CVLs chosen were as similar to each other as possible. From May 2003 to April 2004, 12 samplings were performed in the EVL, and a different CVL was sampled each month for comparison.

Any artifact that was capable of accumulating rainwater was considered to be a breeding site for Culicidae. The breeding sites were classified as follows: potential breeding site (a container with a capacity to accumulate water); actual breeding site (a container with accumulated water); and positive actual breeding site (a container with Culicidae larvae or pupae). The breeding sites were classified into ceramics, metals, organics, plastics, tires and glass.

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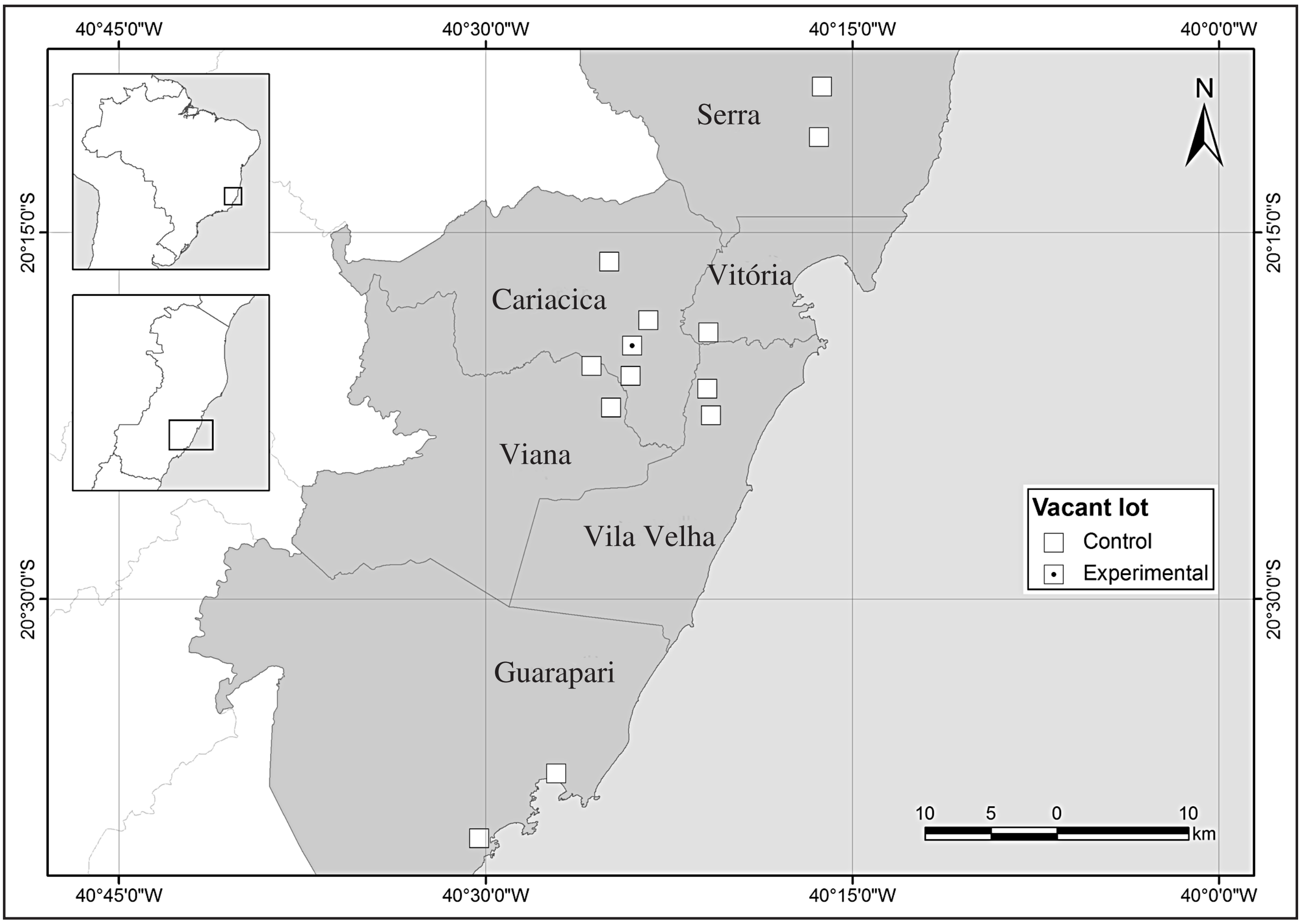

FIGURE 1 - Metropolitan region of Grande Vitória, with squares indicating the sampled vacant lots.

To verify whether the samplings of the CVLs and that of the EVL were comparable, the infestation of *A. aegypti* in the CVLs and in the EVL was monitored using oviposition traps (ovitraps), which were placed after the sampled VLs were cleaned. The premise was, if the indices of *A. aegypti* infestation were statistically similar among post-cleaning CVLs and the EVL, it would be reasonable to infer similar population densities of *A. aegypti* among the different neighborhoods in which the VL samplings were conducted. The VLs were monitored using the ovitraps installed individually at a density of one trap per 100m^2, resulting in the placement of nine traps in each VL. After five days, the oviposition traps were removed and taken to the laboratory. The eggs were exposed to water and, after hatching, the immature forms were identified.

Statistical analysis of the data collected at the EVL and the CVLs was performed using the nonparametric Wilcoxon test for related samples. This test avoids seasonal influence by comparing samples among pairs. The differences in the abundance of the immature forms of *A. aegypti* collected every month from positive actual breeding sites in the EVL and in the CVLs were evaluated. The number of *A. aegypti* larvae that hatched from the eggs collected in the ovitraps installed (after cleaning) in the EVL and in the CVLs were also compared.

In addition, Spearman's nonparametric correlation coefficient was calculated to examine the relation between the number of immature forms of *A. aegypti* verified in the positive actual breeding sites and the average monthly precipitation in the metropolitan region of Greater Vitória [Viana and Vitória Meteorological Stations of the *Instituto Capixaba de Pesquisa, Assistência e Extensão Rural* (INCAPER)]. The same analysis was conducted for *A. aegypti* larvae that hatched from the eggs collected in the ovitraps. For the correlation analysis, the time lag between the collection and the prior influencing events was also taken into account[8]. To factor in this time lag, the correlation between the number of immature forms and the rainfall in the month of sampling was determined, in addition to determining the correlations between the number of immature forms and the rainfall in each of the three months prior to the sampling.

Information on the occurrence (presence/absence) of immature forms in different categories of breeding sites was used to calculate the prevalence ratios of these forms in each breeding site category to estimate the relative risk of the presence of *A. aegypti* in each type of breeding site. This relative risk is defined as the prevalence ratio of *A. aegypti* immature forms in the category concerned (e.g. *metals*) divided by the prevalence ratio of *A. aegypti* immature forms in all the other categories.

Prior to each analysis, the normality assumption of the data was tested using the Shapiro-Wilk test.

A total of 2,300 Culicidae immature forms were collected in actual positive breeding sites, of which 227 belonged to the species *A. aegypti*. Observation confirmed that of these 227, three were from the EVL, while 224 were from the CVLs. A total of 20,241 eggs were collected in the ovitraps. Of the eggs collected at the EVL, 1,256 immature forms of *A. aegypti* hatched, while 733 immature forms hatched from the eggs collected at the CVLs. The *A. aegypti* positive actual breeding sites identified in the CVLs and in the EVL are described in **Table 1**.

TABLE 1 - Description of *Aedes aegypti* positive actual breeding sites found in the experimental vacant lot (submitted to cleaning) and the control vacant lots (not submitted to cleaning) in the metropolitan region of Grande Vitória during the period of May 2003 to April 2004.

Positive actual breeding sites				Immature forms of *Aedes* aegypti			
				experimental vacant lot		control vacant lots	
Date	Category	Description	Water (ml)	larvae	pupae	larvae	Pupae
May/03	metal	Can of paint, capacity 3,600ml, with internal rusted walls	450	-	-	64	-
Jan/04	plastic	Disposable cup, white, capacity 200ml	25	-	-	-	3
Feb/04	plastic	Plastic tarp, 4m long by 40cm wide	4,723	-	3	-	-
Mar/04	tire	Automobile tire	200	-	-	25	-
Mar/04	plastic	Sack of popcorn, opaque, red	10	-	-	2	-
Mar/04	metal	Can of varnish, capacity 3,600ml	510	-	-	72	-
Mar/04	metal	Can of paint, capacity 3,600ml, with internal rusted walls	1,270	-	-	34	-
Mar/04	metal	Can of paint, capacity 3,600ml, with internal rusted walls	630	-	-	16	4
Apr/04	plastic	Sack of dog food, capacity 60kg, opaque, multiple colors	150	-	-	4	-
Total			**7,968**	**-**	**3**	**217**	**7**

The results of the Wilcoxon test revealed that the abundance of *A. aegypti* in positive actual breeding sites was lower in the EVL compared to the CVLs (N = 12; *Z* = -1.63; p = 0.05). However, data collected from the oviposition traps revealed no differences in the abundance of *A. aegypti* in the EVL compared to CVLs (N = 12; *Z* = -0.53; *p* = 0.30), indicating that the VLs were indeed comparable and situated in areas with similar indices of infestation.

Spearman's correlation coefficient, calculated for the number of immature forms of *A. aegypti* collected in positive actual breeding sites and both the average rainfall in the month of sampling (N = 12; r = 0.60; p = 0.02) and in the month prior to sampling (N = 12; r = 0.75; p < 0.01), revealed a statistically significant correlation. In addition, a similar, statistically significant relation was observed between the abundance of *A. aegypti* larvae that hatched from the eggs collected in the oviposition traps and both the average rainfall in the month of sampling (N = 12; r = 0.70; p < 0.01) and in the month prior to sampling (N = 12; r = 0.59; p = 0.02) (**Figure 2**).

Risk analysis was performed by calculating the prevalence ratio of immature forms in various categories of breeding sites, which revealed that the probability of the presence of immature forms was greatest in the Tires category (RR = 79.34; p < 0.01), followed by the Metals category (RR = 35.13; p < 0.01). Analysis also determined that the probability of immature forms was lower in the Plastics category than in the other categories (RR = 0.09; p < 0.01).

Disorganized urban sprawl, a lack of public cleaning services and the indifference of the population itself all promote ample dispersion of containers that accumulate rainwater in VLs[1,4,9]. The present study demonstrates the magnitude of the problem represented by VLs in urban areas. In this study, sampling was performed in CVLs comprising an area of 10,800m^2 and the volume of accumulated rainwater collected from these was greater than 40L; in addition, many more similar VLs are present in these locations that were not sampled.

In their analysis of favorable locations for the propagation of *A. aegypti* in urban areas, Lopes et al[6] concluded that more insect larvae were present in VLs than in auto repair shops, scrap heaps or cemeteries. According to these and other authors, the vegetation available in these areas provides shade for many of the containers that serve as breeding sites, thus making the environment even more favorable for vector growth[6,10].

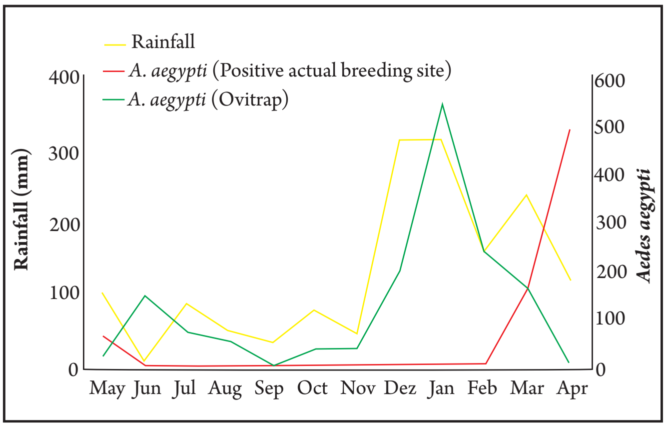

FIGURE 2 - Monthly average precipitation in the metropolitan region of Grande Vitória plotted with the number of immature forms of *Aedes aegypti* collected in actual positive breeding sites and hatched from eggs collected in ovitraps positioned in vacant lots during the time period of May 2003 to April 2004.

The results obtained in the present study demonstrate that monthly cleaning of VLs significantly reduces the abundance of the immature forms of *A. aegypti*. However, it should be taken into account that monthly cleaning will have to counteract the continued deposition of new containers serving as potential breeding sites, owing to the large number of people repeatedly depositing their waste in the VLs[4].

Dengue epidemics generally occur in periods of heavy rainfall[5,11]. During these periods, immature forms of *A. aegypti* are encountered in various containers that are capable of accumulating rainwater, such as vases and plants at cemeteries, swimming pools and native bromeliads[6,12,13]. The positive correlation between precipitation and the abundance of the immature forms of *A. aegypti* collected at positive actual breeding sites and in the oviposition traps indicates that summer rainfall probably accentuates the problem of breeding sites in VLs. Intermittent rainfall, along with elevated temperatures, promotes the greatest emergence and increases the abundance of *A. aegypti*[5].

The high probability of the presence of immature forms of *A. aegypti* in tires confirms the preference of this insect for this type of breeding ground, many of which are located in VLs[14]. Besides tires, metallic containers also offer a relatively high risk of housing *A. aegypti* in VLs. The risk of encountering *A. aegypti* in plastic containers was shown to be considerably lower than in the other categories of breeding sites; however, the harm caused by plastic containers should not be underestimated due to the high number of plastic items discarded by people in VLs.

The natural tendency of *A. aegypti* to disperse widely through a range of habitats is probably facilitated by its ability to deposit its eggs in a wide variety of locations[15]. Control actions aimed at eliminating the preferential breeding sites in residences and in other strategic locations could involuntarily result in forcing the insect to seek alternative locations for procreation. The results presented in this study indicate the need to adopt strategies for combating *A. aegypti*, including the cleaning of VLs, creating awareness among the population regarding the risks associated with discarding waste in inappropriate locations and educating them about the dangers of depositing artifacts capable of accumulating rainwater.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

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