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Short Communication

Immature *Aedes* mosquitoes colonize *Culex quinquefasciatus* breeding sites in neighborhoods in the municipality of Olinda, State of Pernambuco

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ABSTRACT

Introduction: The present study shows the colonization of *Aedes* mosquitoes in breeding sites specific for *Culex quinquefasciatus* in neighborhoods in the municipality of Olinda. **Methods:** Samples were collected between May 2011 and June 2012 from breeding sites positive for *Cx. quinquefasciatus* by using a ladle and manual suction pump. **Results:** *Aedes aegypti* (0.12%), *Aedes albopictus* (0.03%), and *Cx. quinquefasciatus* (99.8%) were found across the breeding sites. **Conclusions:** The presence of *Aedes* ssp. in several *Cx. quinquefasciatus* breeding sites with a heavy load of organic material demonstrates the need to review the concepts and methods used for treatment, as the use of specific larvicide for breeding sites of *Culex*.

Keywords*: Aedes aegypti***.** Colonization. Breeding.

The control of urban mosquito populations, especially those of *Aedes aegypti* Linnaeus (1762), *Aedes albopictus* Skuse (1894), and *Culex quinquefasciatus* Say (1823) (Diptera: Culicidae), is necessary because of the involvement of these species in the transmission of human pathogens such as dengue, yellow fever**,** chikungunya, the Zika virus, filariasis, West Nile virus, and encephalitis, which cause discomfort to human beings and impact quality of life[1,2].

The selection of breeding sites for egg-laying may be associated with factors present in the water, such as organic material, chemical compounds, and the presence of immature*,* among others[3]. Under present conditions, the abundance of egg-laying sites for mosquitoes in cities complicates the monitoring of these insects and makes proper inspection impossible, frustrating efforts to reduce population densities, especially in the case of *Aedes* ssp.

Given the importance of developing new strategies for combating mosquito vectors, the present study aimed to identify the colonization of *Ae. aegypti* in breeding sites positive for *Cx. quinquefasciatus* in neighborhoods with high and low infestation in the municipality of Olinda, which forms part of the Metropolitan Region of Recife, in the Brazilian State of Pernambuco.

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Samples were collected between May 2011 and June 2012. The Municipality of Olinda is located at latitude 8°0'48"S and longitude 34°50'42"W, 6km Northeast of Recife, the capital of Pernambuco. The municipality has an area of 41,681km^2 and an estimated population of 377,779 inhabitants[4]. This region is divided into 33 neighborhoods, grouped into two Sanitary Districts (I and II). The study investigated *Cx. quinquefasciatus* breeding sites located in the neighborhoods of Sanitary District I (*Caixa d'Água, Passarinho, Águas Compridas, Aguazinha, Peixinhos, Alto da Conquista, Alto do Sol Nascente, Alto da Bondade, Sapucaia, São Benedito, Vila Popular, Jardim Brasil, Salgadinho*, and *Sítio Novo*), and strata with high and low infestation with *Ae. aegypti* were selected using the Rapid *Aedes aegypti* Survey Index [LIRAa/2011- Olinda Environmental Surveillance Center - *Centro de Vigilância Ambiental de Olinda* (CEVAO)].

All *Culex quinquefasciatus* breeding sites identified during the study possessed water with organic matter (decomposing plant and animal debris) content and little to no movement. These environmental conditions are conducive to the development of the immature stages of the species[5]. The breeding sites were identified and registered according to location and structural conditions. The density of larvae and pupae (DLP) was estimated using five samples from each site, four in the corners and one in the center, for restricted environments such as tanks. In larger environments, such as ditches and canals, samples were collected only from the edges, with a sample taken every 3m on alternate sides when the site length was between 20 and 50m and every 5m when the site length was over 50m[6]. Samples were initially collected using a ladle, but this apparatus was replaced in the course of the study by a manual suction pump, which could more easily obtain the immature specimens at the bottoms of the breeding sites.

All collected samples were placed in plastic containers (250ml) and taken to the Entomology Laboratory of the Olinda Center for Environmental Surveillance for counting and species identification. In the laboratory, the collected larvae and pupae were placed in test tubes in 70°GL ethanol for identification. The culicids were identified according to external morphological characteristics on a slide under a stereoscopic microscope, using dichotomous keys described in the literature[7]. The numbers of immature individuals at each breeding site were compared using simple analysis of variance (ANOVA). The analyses were made in Statistica 7.0 (StatSoft) at a significance level of 5%.

A total of 11,006 immature culicid specimens were collected, of which 9,129 (82.9%) larvae and 1,861 (16.9%) pupae were of *Cx. quinquefasciatus*, 12 (0.11%) larvae and 1 (0.01%) pupa were of *Ae. aegypti*, and 3 (0.03%) larvae were of *Ae. albopictus* **(Table 1)**.

All breeding sites were positive for *Cx. quinquefasciatus*, including waterholes, canals, channels, drains, junction boxes, inspection tanks, water tanks, and ditches. The numbers of immature *Cx. quinquefasciatus* found in these different types of breeding site did not differ ($p > 0.05$). Four sites tested positive for *Aedes* ssp.: a waterhole, junction box, inspection tank, and ditch **(Table 1)**. The numbers of immature *Ae. aegypti* [$F(7,93) = 3.1570$] and *Ae. albopictus* [$F(7,93) = 3.1570$] were both significantly different ($p < 0.05$) in the different breeding sites. **Table 2** shows that of the 14 neighborhoods inspected, only 4 contained breeding sites that tested positive for *Aedes* ssp. (*Caixa d'Água, Passarinho, Peixinhos*, and *Vila Popular*).

Culex quinquefasciatus, the predominant species found among the breeding sites, prefers to lay its eggs in sites with heavy loads of organic matter, and its genetic and ecological adaptations ensure its greater survival in anthropogenic environments[8].

Aedes aegypti, described as a wild species that originated in Africa, has undergone changes and adaptations in egg-laying behavior over time, as it has accompanied the constant global evolution of human habitation[1]. *Aedes albopictus* is a wild species that originated in Asia, has adapted to the urban environment, and is currently found in many different artificial and natural receptacles[9]. Inadequate disposal of receptacles capable of retaining water has facilitated the development of mosquito groups with the genetic flexibility to develop in these artificial habitats[8].

Aedes aegypti is capable of laying groups of eggs in any receptacle in which water can accumulate[10]. Other biological features of this species, such as its high fertility and fecundity rate, short life cycle, capacity to adapt to environmental changes, and the resistance of its eggs to water, have also contributed to its rapid population growth[11].

The artificial breeding sites for *Ae. aegypti* and *Ae. albopictus* previously described in the literature have included discarded containers, such as cans, bottles, and plastic packaging, and water reservoirs. Natural breeding sites include tree trunks[12] and bodies of water rich in organic material, mainly vegetation[13].

Although junction boxes, inspection tanks, and ditches are not common egg-laying or breeding sites for *Ae. aegypti*, all these habitats tested positive for the species in the present study, as did waterholes for *Ae. albopictus*. These breeding sites are typically dark locations, with little ventilation and a high concentration of volatile organic matter, making them appropriate spots for this opportunistic species to produce offspring. As further proof of the flexibility of the species, Barrera and colleagues[14] found *Ae. aegypti* larvae in septic tanks in Puerto Rico.

During sample collection in the neighborhoods, it was observed that most areas lacked basic sanitation, contributing

TABLE 1 - Total number of larvae and pupae collected from different breeding sites in the 14 neighborhoods of Sanitary District I, Olinda, State of Pernambuco, 2012.

Type of breeding site	Species					
	Aedes aegypti		*Aedes albopictus*		*Culex quinquefasciatus*	
	larvae	pupae	larvae	larvae	pupae	larvae
Waterhole	0	1	3	0	57	25
Canal	0	0	0	0	329	48
Channel	6	0	0	0	6,884	1,369
Drain	0	0	0	0	167	75
Junction box	4	0	0	0	673	176
Inspection tank	1	0	0	0	29	3
Tank	0	0	0	0	561	29
Ditch	1	0	0	0	396	125
Small ditch	0	0	0	0	33	11
Total	12	1	3	0	9,129	1,861

TABLE 2 - Total number of *Aedes* ssp. larvae and pupae collected at different breeding sites located in the neighborhoods of *Peixinhos, Passarinho, Caixa d'Água*, and *Vila Popular*, Olinda, State of Pernambuco, 2012.

			Aedes albopictus		*Aedes aegypti*	
Neighborhood	Type of breeding site	GPS code	larvae	pupae	larvae	Pupae
Peixinhos	Junction box	007	1	0	0	0
	Channel	001	6	0	0	0
	Waterhole	008	0	1	0	0
Passarinho	Junction box	009	3	0	0	0
	Inspection tank	010	1	0	0	0
Caixa d'Água	Ditch	002	1	0	0	0
Vila Popular	Waterhole	004	0	0	3	0

GPS: Global Positioning System.

to the large number of breeding sites testing positive for *Cx. quinquefasciatus*, and that socioeconomic conditions were poor, providing potential breeding grounds for *Ae. aegypti* and leading to a high risk of dengue fever. Ferreira and Neto[15] corroborated the findings of the present study, arguing that factors such as basic sanitation and the cultural and socioeconomic features of communities influence mosquito density and that knowledge of such factors is indispensable for better controlling vectors and reducing the number of dengue fever outbreaks.

Thus, the presence of immature *Ae. aegypti* and *Ae. albopictus* in *Cx. quinquefasciatus* breeding sites demonstrates the need to review the concepts and methods used for treatment of specific larvicide for breeding sites of *Culex*, which may have been rendered obsolete by changes in the behavior of these vectors. The introduction of new measures for monitoring and controlling vectors is thus necessary to ensure the efficiency of the entomological surveillance system.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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