doi: 10.5216/rpt.v53i4.78772

ORIGINAL ARTICLE

Aedes Aegypti AND *Aedes Albopictus* (DIPTERA: CULICIDAE) IN ARTIFICIAL BREEDING SITES IN RURAL SETTLEMENTS AND INDIGENOUS VILLAGES

Paulo Silva de Almeida[1], José Pedro Moreira Carneiro[1], Tamara Nunes Lima-Camara[2], Marcia Bicudo de Paula[2], Eduardo José de Arruda[3] and Herintha Coeto Neitzke-Abreu[4]

ABSTRACT

Aedes aegypti and *Ae. albopictus* are vectors for different arboviruses. *Ae. aegypti* is more common in urban areas, where it uses water collected within artificial receptacles to lay its eggs, *Ae. albopictus* is more prevalent in areas with greater vegetation cover, in which natural water bodies serve as breeding sites for the immature forms. Despite the importance of these two species in Brazil, little is currently known regarding their presence in rural settlements and indigenous villages. In this study, we describe the occurrence of the larvae and pupae of *Ae. aegypti* and *Ae. albopictus* in artificial breeding sites in rural settlements and indigenous villages located in municipalities in Mato Grosso do Sul, Brazil. Collections were carried out in seven rural settlements and two indigenous villages, mainly in 2018. At each location, different households were randomly selected, and the artificial peridomestic containers were inspected for the presence of larvae and pupae. Immature forms were collected at all nine rural locations, and 64 of the 173 households were investigated (37%, 95% CI, 30.04–44.38). A total of 8,744 specimens were collected, among which *Ae. aegypti* was identified as the predominant species, followed by *Culex* spp., *Ae. albopictus*, *Limatus* spp., and *Anopheles* spp. These findings may contribute to developing more effective control strategies for *Ae. aegypti* and *Ae. albopictus* and encourage the maintenance of active and continuous entomological surveillance in these areas, given that these mosquitoes serve as vectors in the transmission cycle of arboviruses with public health importance.

KEY WORDS: insect vectors; arbovirus; mosquitoes control; vector control.

1. Secretaria de Estado de Saúde, Regional Entomology Laboratory, Dourados, Mato Grosso do Sul, Brazil.
2. Universidade de São Paulo, Faculty of Public Health, Department of Epidemiology, São Paulo, São Paulo, Brazil.
3. Universidade Federal da Grande Dourados, Faculty of Exact Sciences and Technology, Dourados, Mato Grosso do Sul, Brazil.
4. Universidade Federal da Grande Dourados, Faculty of Health Sciences, Dourados, Mato Grosso do Sul, Brazil.

Corresponding author: Dr. Paulo Silva de Almeida. E-mail: psilvadealmeida@yahoo.com.br

Paulo Silva de Almeida ORCID: https://orcid.org/0000-0003-2248-8142
José Pedro Moreira Carneiro ORCID: https://orcid.org/0009-0005-7875-7526
Tamara Nunes Lima-Camara ORCID: https://orcid.org/0000-0003-3133-8592
Marcia Bicudo de Paula ORCID: https://orcid.org/0000-0001-5958-8601
Eduardo José de Arruda ORCID: https://orcid.org/0000-0002-9835-467X
Herintha Coeto Neitzke-Abreu ORCID: https://orcid.org/0000-0001-5848-670X

Received for publication: 28/3/2024. Reviewed: 19/6/2024. Accepted: 25/11/2024.

INTRODUCTION

Arboviruses, such as those causing dengue, Zika, and chikungunya, are transmitted worldwide by the mosquitoes *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) and *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1854) (Lounibos & Kramer, 2016). In Brazil, breeding sites for *Ae. aegypti* are predominantly artificial (Consoli & Lourenço-de-Oliveira, 1994), whereas the immature forms of *Ae. albopictus* tends to be more prevalent in natural receptacles that accumulate water (Ferreira-de-Lima et al., 2020).

Entomological surveillance and control of these mosquito vectors are essential to reduce cases of diseases caused by the arboviruses transmitted by these insects. In Brazil, surveillance tends to focus more on *Ae. aegypti* and is carried out according to the Rapid Index Survey for *Ae. aegypti* (LIR*Aa*), the methodology of which entails visiting properties to assess the presence of containers with accumulated water that can serve as breeding grounds for these mosquitoes (Brazil, 2013). If present, the containers are protected or removed, thereby highlighting the preference for mechanical rather than chemical control of the mosquitoes and, consequently, reducing the populations of this species (Brazil, 2013).

In some municipalities in Mato Grosso do Sul, such as Itaquiraí, Itaporã, and Ivinhema, an increase in the incidence of arboviruses in rural populations has been observed over the past decade (SINAN, 2015). Consequently, since the beginning of 2015, the government has developed measures to restructure the health system to better assist the population. Indeed, to the best of our knowledge, no previous collections of mosquitoes have been carried out in these settlement areas and indigenous reserves, thereby emphasizing the need for entomological investigations in these locations.

In this study, we describe the occurrence of the larvae and pupae of *Ae. aegypti* and *Ae. albopictus* in artificial breeding sites within peridomiciliary areas of indigenous villages and rural settlements in five municipalities in the State of Mato Grosso do Sul, Brazil. These findings may contribute to the development of control strategies for these mosquitoes, as well as fostering the maintenance of entomological surveillance in these areas.

MATERIAL AND METHODS

Study area

Larval and pupal surveys were conducted in five municipalities in Mato Grosso do Sul: Amambai (23°06′15″S; 55°13′33″O), Aquidauana (20°28′16″S; 55°47′14″O), Itaporã (22°04′44″S; 51°55′48″O), Itaquiraí (23°28′21″S; 54°10′57″O), and Ivinhema (22°18′17″S; 53°48′55″O) (Figure 1). Most of

the sample collections were conducted in 2018 (Amambai, Aquidauana, and Itaporã), whereas one collection was conducted in 2015 (Itaquiraí) and another in 2021 (Ivinhema).

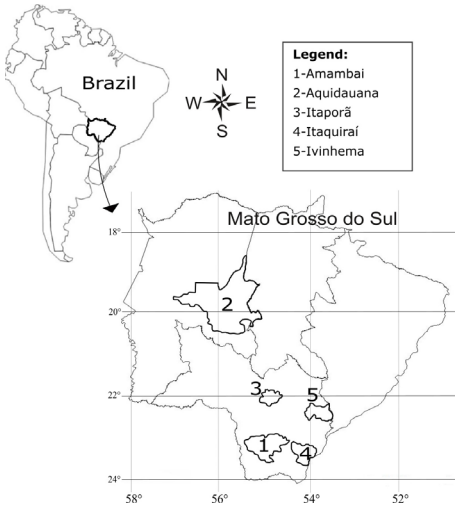

Figure 1. The geographic location of Culicidae in the municipalities of Amambai, Aquidauana, Itaporã, Itaquiraí, and Ivinhema, State of Mato Grosso do Sul, Brazil.

Collections were conducted at nine locations: two in indigenous villages (Village Amambai, 4 km from the center of Amambai, and Village Lagoinha, 90 km from the center of Aquidauana) and seven in rural settlements (São Francisco Site and Passa Frio Farm, located 20 and 15 km from Itaporã, respectively; Indaiá Reservoir 5 km, Santa Rosa 40 km, Santo Antônio 70 km, and Sul Bonito 10 km from Itaquiraí; and Gleba Ouro Verde 11 km from Ivinhema).

Different domiciles were randomly selected for inspection, and in each, all artificial and peridomiciliary containers were inspected for the presence of mosquito larvae and pupae. These locations have particular characteristics, with breeding sites at ground level.

The Amambai and Lagoinha villages are indigenous communities, the former of which belongs to the Amambai Base Pole and has approximately 1,600 residences. The collection of larvae and pupae was carried out in 60 homes from June 12 to 15, 2018. Lagoinha village, which has approximately 150 residences, is in the Taunay Region and belongs to the Aquidauana Base Pole. Larval and pupal collections were carried out in 42 households from April 4 to 5, 2018.

In the municipality of Itaporã, collections of larvae and pupae were carried out in one household at the São Francisco Site (Figure 2) and another at Passa Frio Farm on March 12, 2018. In addition to mining, livestock husbandry, agriculture, and fish farming are the most prominent economic sources of this municipality (IBGE, 2023).

Figure 2. A) Area with the occurrence of *Aedes aegypti* larvae; B) Example of an artificial breeding site (backhoe bucket) where *Ae. aegypti* larvae were collected. São Francisco Site, municipality of Itaporã, Mato Grosso do Sul State, Brazil.

Collections in the municipality of Itaquiraí were carried out in 67 households in four rural settlements (Indaiá, Santa Rosa, Santo Antônio, and Sul Bonito) between March 25 and May 26, 2015. Although most of the regional population is concentrated in urban areas, because of agrarian reform, there has been a significant increase in the rural population in recent years (IBGE, 2023). Collections in this municipality were carried out in two households in the Gleba Ouro Verde settlement on February 10, 2021.

Collections were carried out during the summer and autumn seasons. In this regard, Mato Grosso do Sul State has the following Köppen climatic classification: Am (coldest temperature and short dry season), Aw (rainy season in summer and dry season in winter), Cfa (hot summers, infrequent frosts, and more concentrated rains in the summer) and Af (no defined dry season) (EMBRAPA, 2022).

Collection and identification of larvae and pupae

Collections of larvae and pupae for exploratory analysis were conducted according to the technical standards of the National Dengue Control Program (Brazil, 2002). At each surveyed property/household, mosquito larvae, and pupae were collected from all potential artificial and peridomiciliary breeding sites using plastic pipettes. All immature forms were transferred to small, identified containers with 70% alcohol solution and sent to the Regional

Entomology Laboratory of the State Department of Health, Dourados, Mato Grosso do Sul. Larvae and pupae were identified to the species or genus level using the dichotomous keys proposed by Consoli & Lourenço-de-Oliveira (1994) and Forattini (2002).

Statistical analysis

The proportions of households with mosquito breeding sites were analyzed using OpenEpi version 3.01, with a confidence interval (CI) of 95%.

RESULTS

Mosquito larvae (L3 and L4) and pupae were collected in all nine rural locations evaluated within the five municipalities of Mato Grosso do Sul. Immature forms were present in 64 (37%, 95% CI, 30.04–44.38) of the 173 households analyzed, and a total of 874 specimens were collected from 102 breeding sites (average of 8.57 specimens per breeding site) (Table 1). The predominant species was *Ae. aegypti* (509; 58.2%), followed by *Culex* spp. (248; 28.4%), *Ae. albopictus* (96; 11%), *Limatus* spp. (16; 1.8%), and *Anopheles* spp. (5; 0.6%).

Of the 60 households investigated in Village Amambai, 27 (45%; CI 95%, 32.79–57.68) were positive for immature forms collected from 35 breeding sites. Again, the predominant species was *Ae. aegypti* (152 specimens in 19 breeding sites), followed by *Culex* spp. (88 specimens in 11 breeding sites), *Ae. albopictus* (24 specimens in 3 breeding sites), *Limatus* spp. (8 specimens in 1 breeding site) and *Anopheles* spp. (5 specimens in 1 breeding site) (Table 1).

In Village Lagoinha, in Aquidauana, 22 artificial breeding sites in 12 inspected households were established to be positive for immature forms of mosquitoes, which corresponds to 28.6% (CI 95%, 16.49–43.51) of the total number of households visited. *Ae. albopictus* predominated in this area (72 specimens in 9 breeding sites), followed by *Culex* spp. (64 specimens in 8 breeding sites), *Ae. aegypti* (32 specimens in 4 breeding sites), and *Limatus* spp. (8 specimens in 1 breeding site) (Table 1).

In Itaporã, immature forms were collected from both households visited. At São Francisco Site, 25 specimens of *Ae. aegypti* and 11 *Culex* spp. were collected from one and eight artificial breeding sites, respectively. Similarly, 83 specimens of *Ae. aegypti* were collected from a single breeding site at the Passa Frio Site (Table 1).

In Itaquiraí, 67 households were inspected, and immature forms were collected from 22 (32.8%, CI 95%, 22.41–44.71). *Ae. aegypti* and *Culex* spp. were present in all four evaluated settlements, particularly at the Indaiá Reservoir (82 and 56 immatures, respectively) (Table 1). In Santa Rosa, 41 and

Table 1. Detailed information on immature forms of Culicidae that were collected in rural locations (settlements and Indigenous villages) in municipalities in the State of Mato Grosso do Sul, Brazil.

Municipality	Locality	Collection date	Investigated domiciles	Positive domicile	Species	Number of breeding sites	Number of immatures collected
Amambai	Village Amambai	June 12 to 15, 2018	60	27	*Aedes aegypti*	19	152
					Aedes albopictus	3	24
					Culex spp.	11	88
					Anopheles spp.	1	5
					Limatus spp.	1	8
Total	**-**	**-**	**60**	**27 (45%)**		**35**	**277**
Aquidauana	Village Lagoinha	April 4 to 5, 2018	42	12	*Aedes aegypti*	4	32
					Aedes albopictus	9	72
					Culex spp.	8	64
					Limatus spp.	1	8
Total	**-**	**-**	**42**	**12 (28,58%)**		**22**	**176**
Itaporã	São Francisco Site	March 12, 2018	1	1	*Aedes aegypti*	1	25
					Culex spp.	8	11
	Passa Frio Site		1	1	*Aedes aegypti*	1	83
Total			**2**	**2 (100%)**		**10**	**119**

Itaquiraí	Set.* Indaiá Reservoir	between March 25 and May 26, 2015	42	14	*Aedes aegypti*	14	82
					Culex spp.	8	56
	Set.* Santa Rosa		8	6	*Aedes aegypti*	6	41
					Culex spp.	1	7
	Set.* Santo Antônio		14	1	*Aedes aegypti*	1	6
					Culex spp.	1	8
	Set.* Sul Bonito		3	1	*Aedes aegypti*	1	5
					Culex spp.	1	6
Total		**-**	**67**	**22**		**33**	**211**
Ivinhema	Set. Gleba Ouro Verde	February 10, 2021	2	1	*Aedes aegypti*	1	83
					Culex spp.	1	8
Total	-	-	2	1	-	**2**	**91**
Total	**9**	**-**	**173**	**64**	**5**	**102**	**874**

*Set.= settlements.

seven immatures of *Ae. aegypti* and *Culex* spp., respectively, were identified. In both Santo Antônio and Sul Bonito, only a single household was positive, and in both cases, the immature forms of *Ae. aegypti* and *Culex* spp. were collected from a single breeding site (Table 1).

In Ivinhema, two households were investigated in the Gleba Ouro settlement; one was positive, with a single breeding site containing 83 immature *Ae. aegypti* and eight of *Culex* spp. (Table 1).

DISCUSSION

Although *Ae. aegypti* is a species that is generally closely associated with humans, being found more frequently in urban and peri-urban areas (Consoli & Lourenço-de-Oliveira, 1994; Tauil, 2001; Lima-Camara et al., 2006), our findings in this study revealed the presence of this mosquito at all surveyed sites, which can be considered rural areas. Corroborating our findings, it is expected to find immature forms of *Ae. aegypti* in artificial breeding grounds (Consoli & Lourenço-de-Oliveira, 1994; Pinheiro & Tadei, 2002), including in belowground reuse water reservoirs (Bermudi et al., 2017).

We also collected *Ae. albopictus* from sites in the indigenous villages of Amambai and Aquidauna. This species is frequently found in rural, wild, or peri-urban areas (Braks et al., 2003; Almeida et al., 2006; Lima-Carrara et al., 2006; Honório et al., 2009; Heinisch et al., 2019), in which immature forms are commonly collected from natural breeding sites, such as the water collected in bamboo, tree holes, and bromeliads (Gomes et al., 1992; Marques et al., 2001; Ceretti-Junior et al., 2014). However, the immature forms of *Ae. albopictus* were also collected from tires, water tanks, and potted plants, among other containers (Gomes et al., 1999; Segura et al., 2003; Barbosa et al., 2010; Martins et al., 2010), thereby highlighting that, as observed in the present study, this species can also be found in artificial breeding sites.

In addition to *Ae. aegypti* and *Ae. albopictus*, we also found immature forms of *Culex* spp. in all surveyed areas. Whereas these two *Aedes* species are the main vectors of dengue, Zika, and Chikungunya arboviruses, *Culex* species serve as vectors for the transmission of West Nile and Saint Louis viruses (Heinen et al., 2015; Ciota, 2017). Accordingly, our detection of a high frequency of mosquitoes of this genus requires attention. Despite the small numbers of the immature forms of *Limatus* spp. and *Anopheles* spp. collected in this study, surveillance of mosquitoes in these genera should also be encouraged, given that certain species of *Anopheles*, for example, are involved in the transmission of the etiological agent of malaria (Consoli & Lourenço-de-Oliveira, 1994).

In this study, it was not possible to perform a temporal analysis of the data, and this should be conducted in future studies. Although in this study, it was not feasible to inspect all the artificial breeding sites investigated or to

determine levels of the co-existence of more than one species, our findings highlight the importance of maintaining continuous surveillance of *Ae. aegypti* and *Ae. albopictus*, even in rural areas. Although the Ministry of Health of Brazil has a technical protocol for controlling arboviruses, these measures do not cover rural areas. Despite the closer associations of these two species with man-made and natural environments, respectively, there are reports of the occurrence of *Ae. aegypti* in natural breeding sites and *Ae. albopictus* in artificial breeding sites and/or in more urbanized locations (Varejão et al., 2005; Lima-Carrara, 2016; Ayllón et al., 2017; Medeiros-de-Souza et al., 2020; Multini et al., 2021). Accordingly, the active and continuous surveillance of these species can contribute to the development of more effective control strategies, which may contribute to reducing the number of cases of arbovirus diseases associated with these vectors.

ACKNOWLEDGEMENTS

The authors would like to thank Ezequiel Pereira Ramos and João Nascimento, and other technicians from Vector Control Coordination in the municipalities of Amambai, Aquidauana, Itaporã, Itaquiraí, and Ivinhema for their support in field collections, and the Universidade Federal da Grande Dourados (UFGD) for support to English revision.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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