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Evaluation of the baited ovitrap with natural attractant for monitoring Aedes spp. in Dili, capital of East Timor

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Abstract *Dengue transmission has been known in East Timor since 2005, but the country is not equipped with an Aedes aegypti mosquito monitoring and control program. This study aimed to evaluate the baited ovitrap as a possible tool to monitor the arbovirus vector Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) and was conducted in the city of Dili, capital of East-Timor, between epidemiological weeks 32 (02/08) and 48 (02/12) of 2016. In total, 70 ovitraps were installed in residences scattered throughout fifteen streets of four Administrative Posts (districts) of the city. The following entomological indicators were used: Ovitrap Positivity Index (OPI), Vector Density Index (VDI), and Egg Density Index (EDI). A total of 158.904 eggs were collected during the experiment. The OPI showed that 98-100% of traps contained Aedes spp. in all areas of the study. The EDI and OPI indicators were positively and significantly correlated with the temperature. The two- and three-week lag for rainfall indicated a significant positive correlation for VDI and EDI. Therefore, the ovitrap is a tool that can integrate the actions of an Aedes spp. monitoring and control program in East-Timor.*

Key words *Aedes spp., Oviposition trap, Ovitrap, East Timor*

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Introduction

Aedes mosquitoes originate in the Old World, most likely in northeastern Africa, and were initially described in Egypt[1]. They are considered cosmopolitan mosquitoes and are found in tropical and subtropical areas of the globe, always associated with human domicile and peridomicile[2]. *Aedes* spp. specimens are vectors of several arboviruses such as Dengue, Zika, Chikungunya, and Yellow Fever[3].

Understanding the population dynamics of vectors of medical interest in a given geographic region through vector monitoring allows better planning of public health policies[3,4]. Strategies for monitoring and controlling these insects are necessary to slow down the viral circulation and ensure the population's quality of life[5].

In East Timor, dengue is considered a significant arbovirus transmitted by the *Aedes aegypti*, and studies mainly on vector monitoring[6] are scarce. Given the consecutive cases of dengue in East Timor, from 2014, mainly in the municipality of Dili, it became essential to plan a vector monitoring program through oviposition traps (ovitraps)[7].

The use of ovitraps is a safe, inexpensive, fast, efficient, and environmentally-friendly technique[8]. This tool allows determining the geographic dispersion, density, frequency, occupation, and seasonality of the vector[9].

Methods

Area of study

The municipality of Dili, the capital of East Timor, is located on the north coast of the island, and its bordering municipalities are Manatuto to the east, Aileu to the south, Liquiçá to the west, and the Savu Sea to the north, which also includes the island of Ataúro. The municipality is located at Latitude 8°33'31" S and Longitude 125°34'25" E. The climate is tropical humid with annual mean temperature ranging from 26°C to 32°C and relative air humidity between 60% to 74%.

According to the 2010 census, Dili had a population of 252,884 inhabitants and 39,310 households and an area of 372 km^2. The municipality of Dili is divided into six Administrative Posts: Dom Aleixo, Vera Cruz, Nain Feto, Cristo Rei, Metinaro, and the island of Atauro[10].

Preparation of natural infusion as an attractant of oviposition

The infusion used in the ovitraps was prepared with 350 grams of green leaves of Acacia (*Leucaena leucocephala*) placed in a 15L bucket containing 2L of water. The bucket remained closed for 48 hours to ferment the leaves. After this fermentation period, the infusion was filtered through a clean cloth, and 100 mL of infusion and 400 mL of water were used, totaling 500 mL per oviposition device.

Oviposition Substrate (Palette)

As a substrate for egg-laying, we used fabric (80% cotton and 20% polyester, made in China), light brown, and measuring 12 cm long by 6 cm wide. The tissue had a smooth side and a rough side, and the rough side was exposed for oviposition of female *Aedes* spp. The palettes were placed vertically and attached by a clip inside the device.

Installation of ovitraps

Seventy ovitraps were installed in 15 streets from four Dili Administrative Posts. The selection of Administrative Posts for the study took into consideration the availability of human resources, and we selected the Administrative Posts of Dom Aleixo (36 ovitraps), Cristo Rei (8 ovitraps), Nain Feto (11ovitraps) and Vera Cruz (15 ovitraps). The traps were allocated by convenience in the buildings, with 64 devices installed in the intra-domicile area (47 in the bedroom, 12 in the kitchen, 5 in the bathroom), and six in the peridomicile of the property (on the balcony). The facilities and surveys of the devices were handled by the students of the National University of Timor Lorosa'e.

The ovitraps were regularly inspected every seven days for palette change and infusion during the 17 weeks of study. The structure of the laboratory of the Department of Biology of the Faculty of Education, Arts, and Humanities of the National University of Timor Lorosa'e was used for counting eggs of *Aedes* spp. in the palettes.

Meteorological data

Meteorological data for mean, minimum, maximum temperature, rainfall, and relative humidity of the municipality of Dili were provided by the Ministry of Public Works of East Timor.

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Data analysis

The eggs collected in the ovitraps allowed to calculate the total eggs of *Aedes* spp., the Ovitrap Positivity Index (OPI = *Aedes* spp. egg trap percentage and installed traps), Egg Density Index (EDI = *Aedes* spp. eggs and positive traps) and the Vector Density Index (VDI = *Aedes* spp. eggs and the traps inspected)[11].

The OPI, VDI, EDI, and total egg data of *Aedes* spp. were correlated with abiotic factors (maximum, mean, minimum temperature, rainfall, and relative humidity) using Pearson's test. The VDI per Epidemiological Week was submitted to the Shapiro-Wilks normality test at 5% probability, showed a normal distribution, and underwent analysis of variance (Anova). The entomological means were compared *a posteriori* by the Tukey test at the significance level of $p < 0.05$.

Data were processed using the following programs: IBM SPSS version 2.4, and Microsoft® Office 365™ Excel and Word programs.

Results

During the 17 weeks of study, a total of 158,904 eggs of *Aedes* spp. in the four Administrative Posts of Dom Aleixo, Cristo Rei, Nain Feto, and Vera Cruz from the Dili capital of East Timor.

The largest proportion of *Aedes* spp. eggs (62.5%; 99,317 eggs) was recorded in Dom Aleixo, followed by Cristo Rei (16%; 25,396 eggs), Nain Feto (12%; 19,056 eggs) and Vera Cruz had the lowest proportion (9.2%; 14,585 eggs).

The mean number of *Aedes* spp. eggs showed no significant difference by oviposition installation environment in Dili residences (Anova $F_{(4, 289)} = 0.678$; $p > 0.05$) where in the intra-domicile environment represented by the bedroom collected on average 129 eggs, in the kitchen, 141 eggs; in the bathroom, 146 eggs; and in the peridomicile environment represented by the balcony, 134 eggs of *Aedes* spp.

The indicators provided by ovitraps evidenced that the VDI and EDI showed similar behavior in the four evaluated Administrative Posts, where Dom Aleixo ranged from 109.6 to 199.3; Cristo Rei, from 93 to 134.4; Nain Feto ranged from 73.4 to 143.4; and Vera Cruz ranged from 85.5 to 129.4. Noteworthy is the Dom Aleixo Administrative Post, with EDI above 100 eggs of *Aedes* spp. throughout the experimental period and peaking at 199 eggs at Epidemiological Week 43 (Table 1).

The VDI in the municipality did not observe a significant difference during the experimental period (Anova, $F_{(17,68)} = 1.03$; $p > 0.05$) (Figure 1).

The OPI at Dom Aleixo, Nain Feto, and Vera Cruz Administrative Posts ranged from 98.03 to 100%, and the Cristo Rei Administrative Post presented 100% of the positive egg traps of *Aedes* spp. During the study, mean, minimum, and maximum temperatures ranged from 26 to 32°C, 16 and 24°C, and 28 and 35°C, respectively. Large fluctuations were observed for weekly rainfall, between August and September (EW 32 to 39) of 2016, a mean rainfall of 0.21mm occurred, while during the remaining epidemiological weeks, a mean of 1.53mm of rainfall representing the rainy season from October to December (Figure 2). The mean weekly relative air humidity ranged from 58% to 72%.

Pearson's correlation analysis indicated a positive and significant relationship between total eggs, VDI, and EDI with the maximum, mean, and minimum temperature variables for the study period. No significant correlation was observed in the entomological variables provided by ovitrap and correlated with relative air humidity and precipitation in the same week. Relative humidity was negatively correlated with VDI, OPI and EDI, and rainfall for total eggs and OPI (Table 2).

However, the entomological indicators provided by ovitrap, when correlated with rainfall, under the effect of one, two, three and four weeks' lag, observed that the action of delaying in two ($r^2 = 0.518$, $p = 0.0332$) and three weeks ($r^2 = 0.5034$, $p = 0.0394$) showed a significant positive correlation for the VDI and EDI variables (Table 3).

Discussion

The ovitrap has been used in several countries to monitor *Aedes* spp.[8,9,11]. In the municipality of Dili, the ovitrap proved to be a highly sensitive tool for monitoring Aedes infestation, providing data at short (weekly) intervals and continuously.

The weekly monitoring highlighted the Dom Aleixo Administrative Post with 62.5% of the eggs collected compared to the Cristo Rei, Nain Feto, and Vera Cruz Administrative Posts. The high percentage of eggs collected at Dom Aleixo Administrative Post can be attributed to the fact that it is a more urbanized area of the city of Dili with disordered growth and irregular water sup-

Table 1. Entomological variables provided by the ovitrap trap in the city of Dili, Timor-Leste, from August to December 2016 (EW 32 to 48).

Epidemiological Week	Entomological Variables											
	Dom Aleixo			Cristo Rei			Nain Feto			Vera Cruz		
	VDI	EDI	OPI	VDI	EDI	OPI	VDI	EDI	OPI	VDI	EDI	OPI
32	116.5	116.5	100	94.9	94.9	100	84.9	84.9	81.8	95.9	95.9	100
33	133.9	133.9	100	93	93	100	106.6	106.6	100	93.7	93.7	100
34	123.3	123.3	100	105.8	105.8	100	83.6	83.6	100	86.7	86.7	93.3
35	129.5	129.5	97.2	95.3	95.3	100	81.5	81.5	100	95.7	95.7	93.3
36	109.6	109.6	100	93.1	93.1	100	82.5	82.5	100	93.5	93.5	93.3
37	119.8	119.8	100	98.6	98.6	100	82.1	82.1	100	93.5	93.5	100
38	141.2	141.2	100	95.3	95.3	100	73.4	73.4	100	94.6	94.6	100
39	153.7	153.7	100	106.9	106.9	100	94.4	94.4	100	96.1	96.1	100
40	191.3	191.3	100	109.4	109.4	100	106.2	106.2	100	85.5	85.5	100
41	191.7	191.7	100	101.5	101.5	100	97.4	97.4	100	112.1	112.1	100
42	194.7	194.7	100	99.6	99.6	100	109.2	109.2	90.9	105	105	100
43	199.3	199.3	100	108.8	108.8	100	100.2	100.2	100	108.4	108.4	100
44	196.3	196.3	100	126.1	126.1	100	99.7	99.7	100	105.5	105.5	100
45	190.4	190.4	100	122.9	122.9	100	115.4	115.4	100	105.3	105.3	100
46	194	194	100	118.6	118.6	100	133.3	133.3	100	97.5	97.5	86.7
47	191	191	100	120.4	120.4	100	138.5	138.5	100	94.8	94.8	100
48	182.7	182.7	100	134.4	134.4	100	143.4	143.4	100	129.4	129.4	100

Captions: VDI = Vector Density Index; EDI = Egg Density Index; OPI = Ovitrap Positivity Index.

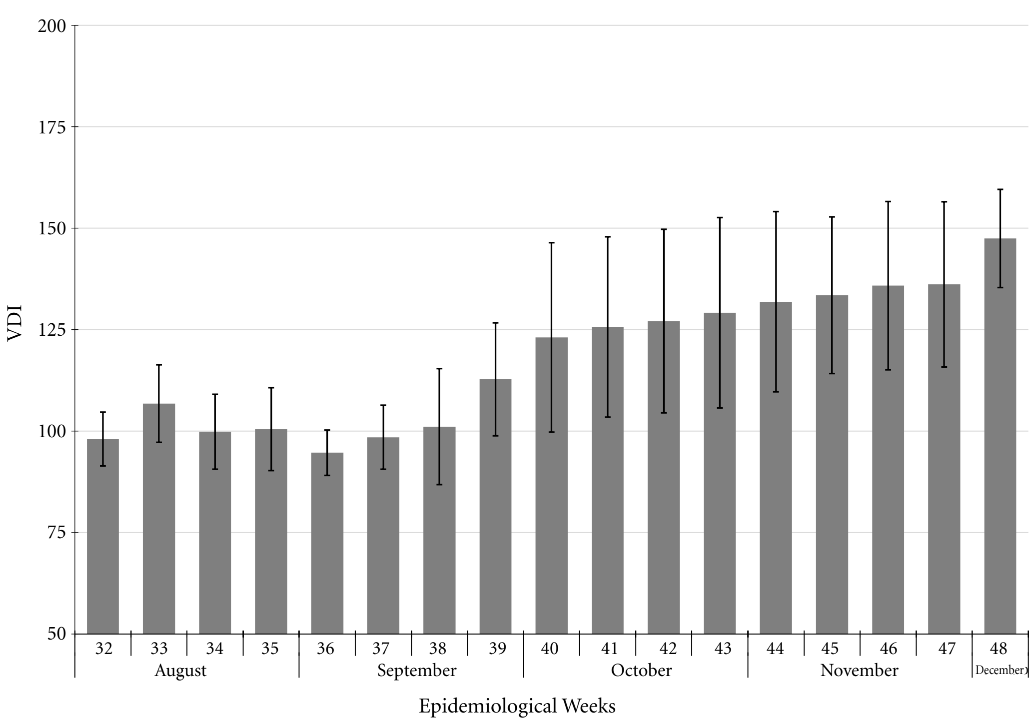

Figure 1. Vector Density Index (μ ± standard error) of Aedes spp. per epidemiological week in the city of Dili, the capital of Timor-Leste, from August to December 2016.

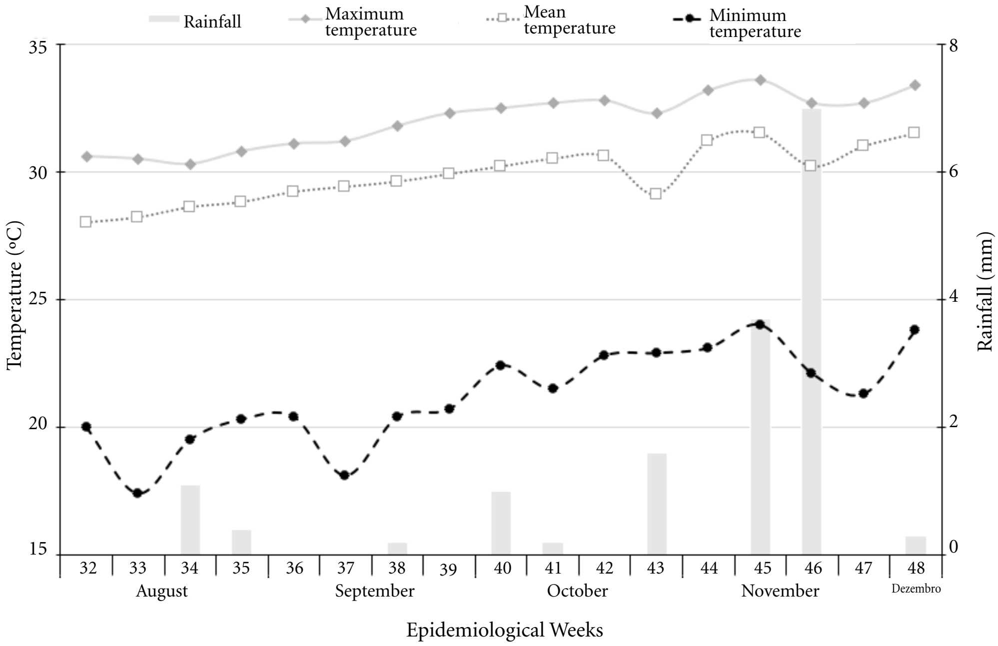

Figure 2. Weekly weather variables in the city of Dili, capital of Timor-Leste, from August to December 2016.

Table 2. Pearson's correlation between abiotic variables in total eggs collected, VDI, OPI, and EDI in the city of Dili, Timor-Leste, from August to December 2016.

Abiotic variables		Entomological Variables			
		Total eggs	VDI	OPI	EDI
		Corr.Coeff. (p)	Corr.Coeff. (p)	Corr.Coeff. (p)	Corr.Coeff. (p)
T	Max.	0.907 (<0.0001)*	0.8736 (<0.0001)*	0.1876 (0.4708)	0.8736 (<0.0001)*
	Mn.	0.805 (<0.0001)*	0.7954. (0.0001)*	0.176 (0.4993)	0.7954. (0.0001)*
	Min.	0.827 (<0.0001)*	0.7879 (0.0002)*	0.2178 (0.4011)	0.7879 (0.0002)*
RH	Mn.	0.369 (0.1444)	(-)0.2186 (0.3993)	(-)0.3177 (0.2140)	(-)0.2186 (0.3993)
RF	Mn.	(-)0.2208 (0.3945)	0.4775 (0.0526)	(-)0.1303 (0.6181)	0.4775 (0.0526)

Acronyms: T = Temperature; Max. = Maximum; Mn. = Mean; Min. = Minimum; RH = Relative humidity of air; RF = Rainfall. * Significant correlation between variables.

Table 3. Pearson's correlation between the rainfall meteorological variable and total eggs collected, VDI, OPI, and EDI in the city of Dili, Timor-Leste, from August to December 2016.

Weekly lag	Entomological Variables			
	Total eggs	VDI	OPI	EDI
	Corr.Coeff. (p)	Corr.Coeff. (p)	Corr.Coeff. (p)	Corr.Coeff. (p)
1 week	0.3668 (0.1476)	0.4321 (0.0832)	(-) 0.0977 (0.7091)	0.4321 (0.0832)
2 weeks	0.4113 (0.1010)	0.518 (0.0332)*	0.01647 (0.9500)	0.518 (0.0332)*
3 weeks	0.3606 (0.1550)	0.5034 (0.0394)*	(-) 0.129 (0.6218)	0.5034 (0.0394)*
4 weeks	0.2253 (0.3847)	0.1836 (0.4805)	(-) 0.03193 (0.9032)	0.1836 (0.4805)

* Significant correlation between variables.

ply system, probably contributed to the higher prevalence of *Aedes* spp. in this area[12].

When analyzing the environments for the installation of ovitraps, no significant differences were found in *Aedes* spp. collected in the ovitraps, installed in the intra-domicile environment (bedroom, kitchen, and bathroom) or the home (balcony). Despite not observing a difference between the analyzed environments, the high proportion of female *Aedes* spp. that do posture on breeding grounds within buildings. The event is due to poor housing conditions in Dili, houses built with openings in the walls allowing free movement of *Aedes* mosquitoes[7]. These results differ from the Brazilian findings, where most of *Aedes* spp. are found in the peridomicile area[12,13].

The weekly VDI of *Aedes* spp. provided by the ovitrap in Dili (East Timor) did not show significant differences, with a higher and lower infestation, during the experimental period.

The ovitrap's OPI allowed the qualitative assessment of the presence of *Aedes* spp., showing that the Dom Aleixo, Cristo Rei, Nain Feto, and Vera Cruz Administrative Posts were similar and ranged from 98 to 100%. Despite not having the highest EDI and VDI, the Cristo Rei Administrative Post traps remained positive throughout the experiment. This positivity may be associated with the female *Aedes* spp. behavior, which does not lay all its eggs in a single breeding place, distributing them in different containers[13]. This behavior, called "skip oviposition", allowed the same female to lay eggs in more than one trap[14].

The OPI data in the administrative posts of the city of Dili allowed indicating that the studied areas are at high risk for the transmission of dengue and other arboviruses by *Aedes aegypti* and *Aedes albopictus*. According to the literature, the OPI indicator equal to or greater than 40% of positivity indicates the risk of arbovirus transmission[11].

Aedes spp. EDI and VDI by Dili Administrative Post varied similarly, and in most epidemiological weeks, were higher than 100 eggs of *Aedes* spp. collected by positive and inspected ovitraps, respectively. This behavior of EDI and VDI indicators is attributed to the high rate of *Aedes* spp.[13,14] population infestation found in the evaluated Administrative Posts.

The high rates provided by ovitraps in the city of Dili may be associated with disordered population growth as a consequence of the civil war that brought imbalances in urbanization[10]. It is observed that the water supply in the city is irregular and the absence of a sewage collection system, where residential cloacal waste remains in the open, characteristics of occupation of the urban area that stimulate the proliferation and dispersion of *Aedes* spp. and other culicides[12,13].

Regarding abiotic variables and infestation indices of *Aedes* spp. in Dili, a significant correlation was found between the VDI, EDI indicators, and the total number of *Aedes* spp. eggs collected with the temperature. No positive association was observed between rainfall in the same epidemiological week and relative air humidity. Rainfall was positively correlated with VDI and EDI indicators only when data were lagged at two and three weeks. This time lag probably allowed adjusting the rainfall variable as per the biological cycle of *Aedes aegypti* and *Aedes albopictus*. This time of two and three weeks is related to the appearance of potential breeding after the rainy season[14].

The positive correlation data with the temperature that acts as a model factor for the *Aedes* spp. infestation process is in agreement with the results of the specific literature[15,16], which emphasized the importance of temperature on the distribution of *Aedes* spp. East Timor climatic seasons can be divided into two periods, the dry season that occurs between May and October, and the rainy season that occurs between November and April.

Climatic conditions are determining factors for the existence and maintenance of *Aedes aegypti* in the environment[16]. Thus, the ideal temperature for the proliferation of the Aedes aegypti mosquito would be between 24ºC and 28ºC, and the ideal relative air humidity above 70%. Under these conditions, the *Aedes aegypti* reproduces with higher intensity, increases its survival, oviposition, hematophagic activity, and the efficiency of virus reproduction in its environment[15]. These weather patterns are found to have occurred throughout the experimental period in Dili.

Therefore, this study suggests the use of ovitraps in Dili, East Timor, as an appropriate method for detecting the presence and population density variation of *Aedes aegypti* in different environments. Therefore, the Ministry of Health of East Timor will be able to use the indicators provided by the ovitrap to direct *Aedes* spp. control activities, enabling it to act more effectively in the positive trap coverage areas and areas with EDI and VDI values indicating the risk of arbovirus transmission by *Aedes* spp.

Collaborations

E Barreto: Organization of field tests, training, and orientation of undergraduate students who participated in the project, tabulation of previous results. MC Resende: Elaboration of field test protocols, monitoring of field tests, elaboration of study discussions, and tabulation of final results. AE Eiras: Research Coordinator, worked on the elaboration of field protocols, field test follow-up, final reviewer. PC Demarco Júnior: Preparation and standardization of written material, preparation of statistical tests, and results.

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