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Monitoring the density of *Aedes aegypti* (Diptera: Culicidae) through ovitrap in the urban area of Manaus – Amazonas, Brazil

Monitoramento da densidade de *Aedes aegypti* (Diptera: Culicidae) através de ovitrampa na área urbana de Manaus – Amazonas, Brasil

Monitoreo de la densidad de *Aedes aegypti* (Diptera: Culicidae) mediante ovitrampa en el área urbana de Manaus – Amazonas, Brasil

Aylane Tamara dos Santos Andrade[1], André Correa de Oliveira[2], Hergen Vieira de Souza[2], Vinicius Braz Ribeiro[3], Joelma Soares da Silva[4], Valéria Cristina Soares Pinheiro[5], Rosemary Aparecida Roque[2].

ABSTRACT

Objective: To compare two monitoring methods, oviposition traps and *Aedes aegypti* Infestation Index Rapid Survey (LiRAa), aiming to analyze which method is more effective for estimating *A. aegypti* population density. **Methods:** Comparative study between LiRAa and population density obtained through 600 oviposition traps from August to September 2021 in the municipality of Manaus/AM. **Results:** 12.211 *Aedes* eggs were collected. Of these, (12.96%) in the North health district, followed by the East (23.16%), South (28.44%) and West (35.44%) districts. A difference was identified only between the North and West districts ($p < 0.05$). When analyzing LiRAa in the four health districts, 3.401 homes were inspected. Of these, (42.99%) in the West Zone, followed by the East Zone (38.25%), North (12.73%) and South (6.03%). When checking the Breteau Index, the unpaired *t* test did not identify a statistical difference in the level of infestation of immature *Aedes*. **Conclusion:** Oviposition traps and LiRAa are important tools for monitoring the infestation of mosquitoes of the genus *Aedes*. However, ovitraps can provide more accurate data on vector dispersal, which allows greater agility in controlling *A. aegypti*, the main transmitter of arboviruses in the country.

Keywords: Vectors, Breeding sites, Entomological surveillance.

RESUMO

Objetivo: Comparar dois métodos de monitoramento, armadilhas de oviposição e Levantamento Rápido de Índices para o *Aedes aegypti* (LiRAa), visando analisar qual método é mais eficaz para estimar a densidade populacional de *A. aegypti*. **Métodos:** Estudo comparativo entre LiRAa e densidade populacional obtida através de 600 armadilhas de oviposição no período de agosto a setembro de 2021 no município de Manaus/AM. **Resultados:** Foram coletados 12.211 ovos de Aedes. Destes, (12,96%) no distrito sanitário Norte, seguido pelos distritos Leste (23,16%), Sul (28,44%) e Oeste (35,44%). Foi identificada diferença apenas entre os distritos Norte e Oeste ($p < 0,05$). Ao analisar o LiRAa nos quatro distritos sanitários, foram fiscalizados 3.401 domicílios. Destas, (42,99%) na Zona Oeste, seguida pela Zona Leste (38,25%), Norte (12,73%) e Sul (6,03%). Ao verificar o Índice de Breteau, o teste t não pareado não identificou diferença estatística no nível de infestação de *Aedes* imaturos. **Conclusão:** Armadilhas de oviposição e LiRAa são

[1]Programa de Pós-Graduação - Rede de Biodiversidade e Biotecnologia da Amazônia Legal/Bionorte, Manaus - AM.
[2]Instituto Nacional de Pesquisas da Amazônia- INPA, Manaus - AM.
[3]Escola Superior Batista do Amazonas-ESBAM, Manaus - AM.
[4]Universidade Federal do Maranhão-UFMA, Campus Codó - MA.
[5]Universidade Estadual do Maranhão-UEMA, Campus Caxias - MA.

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importantes ferramentas para monitoramento da infestação de mosquitos do gênero *Aedes*. Porém, as ovitrampas podem fornecer dados mais precisos sobre a dispersão do vetor, o que permite maior agilidade no controle do *A. aegypti*, principal transmissor de arboviroses no país.

Palavras-chave: Vetores, Criadouros, Vigilância entomológica.

RESUMEN

Objetivo: Comparar dos métodos de monitoreo, trampas de oviposición y Encuesta Rápida del Índice de Infestación de *Aedes aegypti* (LiRAa), con el objetivo de analizar cuál método es más efectivo para estimar la densidad poblacional de *A. aegypti.* **Métodos:** Estudio comparativo entre LiRAa y densidad poblacional obtenida a través de 600 trampas de oviposición de agosto a septiembre de 2021 en el municipio de Manaus/AM. **Resultados:** Se recolectaron 12.211 huevos de *Aedes.* De ellos, (12,96%) en el distrito de salud Norte, seguido por los distritos Este (23,16%), Sur (28,44%) y Oeste (35,44%). Sólo se identificó una diferencia entre los distritos Norte y Oeste ($p < 0,05$). Al analizar LiRAa en los cuatro distritos sanitarios, se inspeccionaron 3.401 viviendas. De ellos, (42,99%) en la Zona Oeste, seguida de la Zona Este (38,25%), Norte (12,73%) y Sur (6,03%). Al verificar el índice de Breteau, la prueba t no apareada no identificó una diferencia estadística en el nivel de infestación de *Aedes* inmaduros. **Conclusión:** Las trampas de oviposición y LiRAa son herramientas importantes para monitorear la infestación de mosquitos del género Aedes. Sin embargo, las ovitrampas pueden proporcionar datos más precisos sobre la dispersión de vectores, lo que permite una mayor agilidad en el control de *A. aegypti*, principal transmisor de arbovirus en el país.

Palabras clave: Vectores, Sitios de reproducción, Vigilancia entomológica.

INTRODUCTION

The species *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) and *Aedes (Stegomyia) albopictus* (Skuse, 1894) are considered relevant due to the vectorial capacity of females to transmit different etiological agents that can cause diseases such as urban yellow fever, chikungunya, Zika and dengue (ZAYED A, et al., 2012; WHO, 2022). Among them, dengue is the arbovirus with the highest global incidence, with around 390 million people infected by the virus, being endemic in more than 100 countries in the regions of Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific (WHO, 2023).

Countries in the Americas are seriously affected by the incidence of dengue cases (WHO, 2023). In Brazil, in 2023, around 1,530.940 cases of dengue (946 deaths), 143.739 of chikungunya (82 deaths) and 8.425 of Zika (no deaths) were recorded, which are transmitted by the primary vector *A. aegypti*, which is found in the country has high levels of rainfall and temperatures that determine vector density, as well as *A. albopictus* (SOARES-PINHEIRO VC, et al., 2017; DICKENS BL, et al., 2018; KAMAL M, et al., 2018; MS, 2023).

In addition to the factors mentioned above, the population density of mosquitoes is attributed to the adaptive capacity of *A. aegypti* and *A. albopictus* females laying eggs in any containers that can accumulate water (ZAYED A, et al., 2012; WHO, 2022), especially those used by residents inside or outside homes to store water (SOARES-DA-SILVA J, et al., 2012; SANOUSSI AF, et al., 2015; HASHIM NA, et al. 2018, ANDRADE ATS, et al., 2019) which may or may not have conspecific larvae and heterospecific (GONZALEZ PV, et al., 2015).

With the abundance of specimens in domestic containers and the favorable conditions for the population expansion of the vectors, the Ministry of Health determined that in addition to the mechanical removal of breeding sites, new simplified sampling methods, such as monitoring by the Rapid Index Survey for *Aedes aegypti* – LiRAa (MS, 2013), was implemented to obtain data through systematic and periodic surveys, used to monitor the population density of *A. aegypti* with an acceptable percentage of errors and in a simple, quick and economical way (MS, 2013).

In addition to LiRAa, another more rapid and cost-effective monitoring approach can be used, such as the installation of oviposition traps (ovitraps) that consist of dark-colored plastic containers, with an opening approximately 12 cm wide in the upper part where it is a cardboard paddles type is vertically fixed, 12 cm x 2.5

cm wide and with a capacity of 800 mL (FAY RW e ELIASON DA, 1966), that simulate conditions for egg laying (SILVA WR, et al., 2018).

Ovitraps consist of artificial containers containing water and/or appropriate substrate, such as a rough surface or floating material, which serve as a place for egg laying. These structures simulate ideal conditions for egg laying by mosquitoes (SILVA WR et al., 2018). When associated with an attractive solution, these traps are enhanced and become even more conducive for females to lay their eggs, which makes it possible to monitor the abundance and distribution of these insects in the environment. These solutions may include chemical substances or natural compounds that attract females to lay their eggs there. Therefore, ovitraps offer an efficient way to monitor the abundance and distribution of these mosquitoes in a given geographic area (DEPOLI PAC et al., 2016; SILVA WR et al., 2018).

The use of ovitraps stands out as an essential tool used in vector control initiatives, allowing the collection of data that helps understand reproduction patterns and implement targeted strategies to reduce the population of disease-transmitting mosquitoes. This approach contributes to epidemiological surveillance and the development of more effective preventive measures to combat diseases transmitted by these insects (SILVA WR, et al., 2018).

Given the importance of oviposition for the dispersion and maintenance of mosquitoes in the environment, the implementation of new approaches to validate the monitoring of females is essential to develop surveillance measures for mosquito vectors. Furthermore, considering that there is not just one specific method to estimate the population density of these insects, comparative studies are necessary in order to evaluate the different methods of monitoring *A. aegypti.*

In this sense, the present study aimed to verify the presence and abundance of *A. aegypti* mosquitoes in the four health districts of the city of Manaus/AM, using oviposition traps, which represent a specific and sensitive approach to collecting data on the reproductive activity of mosquito vectors and compare the positivity rates and egg density with those of the *A. aegypti* Infestation Index Rapid Survey (LIRAa).

In order to expand the understanding of the effectiveness of these monitoring tools. Since the correlation between these indices can provide crucial information to improve control and prevention strategies, allowing a more efficient response to local epidemiological conditions. Therefore, this study aims to contribute to a more comprehensive and precise approach to tackling public health threats associated with the presence of *A. aegypti.*

METHODS

Study area

The study was carried out in the North, South, East and West health districts where ovitraps were installed to capture eggs, in six neighborhoods in the city of Manaus - Amazonas, in the months of August and September 2021 (SISBIO/79015-1/2021 -2022). The traps were installed with the support of endemic disease agents from the Department of Environmental and Epidemiological Surveillance of the Municipality of Manaus, after authorization from the resident, upon signing the Free and Informed Consent Form (TCLE).

Were used 600 ovitraps during five consecutive weeks. The cardboard paddles, oviposition substrate, in the traps were replaced every five days until the last week of collection. Subsequently, the cardboard paddles containing the eggs were transported to the Malaria and Dengue Biological Control and Biotechnology Laboratory of the National Institute of Amazonian Research and kept under controlled conditions of temperature (27 ± 2 °C), humidity (80 ± 5%) and photoperiod (12 Light:12 Dark).

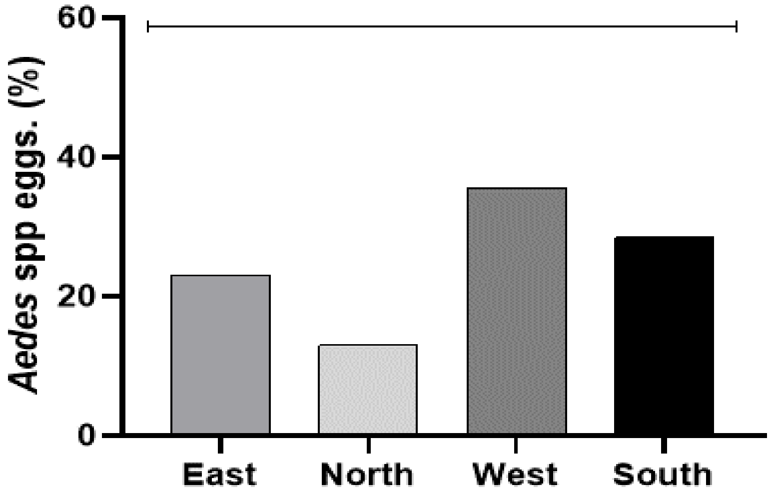
Statistical analysis

The Positivity Ovitraps Index - POI (POI = Number of positive traps / Number of traps inspected) x 100 and the Egg Density Index - EDI (EDI = Number of eggs / Number of positive traps) were calculated (GOMES AC, 1998). For the analyzes of the *Aedes aegypti* Rapid Index Survey (LiRAa), the Breteau Index –BI (Positive containers/Properties surveyed × 100) and the Building Infestation Index –PI (Positive properties/Properties surveyed × 100) were calculated. The difference between the POI and EDI indices compared to the BI and PI was analyzed using the student's *t* test ($p \leq 0.05$). Kruskal-Wallis test ($p \leq 0.05$) was applied to identify the quantitative difference between eggs collected during the five weeks, as well as between districts. Graphic Prism software version 9 (GraphPad Software, 1989) was used in all analyses.

RESULTS

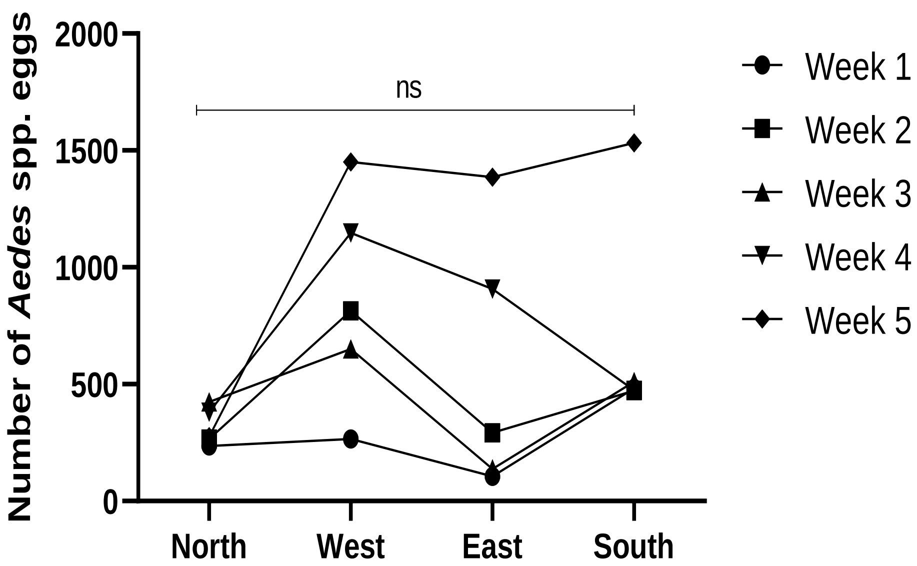
Of the total of 600 traps installed, 233 (38.8%) were positive, collecting a total of 12.211 *Aedes* eggs. Of these, 1.583 (12.96%) were collected in the North district, followed by the East district 2.828 (23.16%), South 3.473 (28.44%) and West 4.327 (35.44%). However, there was statistically significant difference between the four health districts sampled (**Figure 1**). When the number of eggs collected each week was compared, no significant statistical difference was observed ($p > 0.05$) (**Figure 2**), using the Kruskal-Wallis test.

Figure 1 - Percentage of *Aedes* spp. eggs collected in the four health districts, from August to September 2021, in the city of Manaus - Amazonas.



Source: Andrade ATS, et al., 2024.

Figure 2 - Number eggs of *Aedes* spp. eggs. collected during five consecutive weeks, from August to September 2021, in the city of Manaus - Amazonas.



Source: Andrade ATS, et al., 2024.

In relation to the Positivity Ovitrap Index (POI), 49.3% of positive traps were registered in the West district, followed by the East (36.64%) and South (35.96%) districts, while the lowest index was registered in the North district (23.98).

A significant statistical difference was only recorded between the North and West districts ($p < 0.05$). The highest value of the Egg Density Index (EDI) was observed in the West district (54.83) while the EDI observed for the other districts evaluated did not have major variations, with an EDI of 44.14 being observed for the North district, 42.44 for the East and 42.02 for the South. However, no statistically significant difference ($p > 0.05$) was observed between the EDI of the four health districts evaluated (**Table 1**).

Table 1 - Positivity Ovitraps Index -POI and Egg Density Index - EDI of *Aedes* spp. collected during five consecutive weeks from August to September 2021 in Manaus, Amazonas, Brazil.

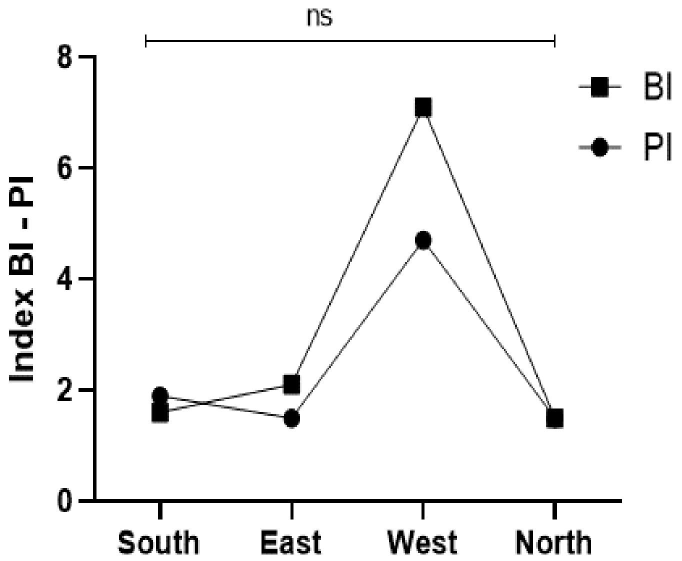
Weeks	Health Districts							
	North		South		East		West	
	POI (%)	EDI	POI (%)	EDI	POI (%)	EDI	POI (%)	EDI
1	20	39.3	46.6	34.5	16.6	21	30	29.4
2	20	44.3	16.6	39.2	33.3	29.2	50	54.2
3	26,6	53	30	50.2	30	15.2	46.6	46.4
4	33,3	38.1	46.6	44.7	43.3	69.8	53.3	71.6
5	20	46	40	41.6	60	77	66.6	72.55
Average	23.98	44.14	35.96	42.04	36.64	42.44	49.3	54.83

Source: Andrade ATS, et al., 2024.

During the *A. aegypti* rapid index survey in the four health districts, 3.401 homes were inspected, of which 198 (5.82%) were positive for *A. aegypti*. The highest percentage of homes inspected was in the West district 1.462 (42.99%), followed by East 1.301 (38.25%), North 433 (12.73%) and South 205 (6.03%).

When analyzing the Breteau Index and Building Infestation Index in the health districts, the lowest rates for both analyzes were recorded in the North district (BI and PI =1.5), the South district presented BI (1.6) and PI (1.9), followed by the East district with BI (2.1) and PI (1.9), while the highest number was found in the West district with (7.1) and (4.7) respectively. However, no significant difference was found when comparing the BI and PI values of each health district ($p > 0.05$) (**Figure 3**).

Figure 3 - Breteau Index (BI) and Building Infestation Index (PI) obtained from August to September 2021 in Manaus - Amazonas.



Source: Andrade ATS, et al., 2024.

DISCUSSION

The *Aedes aegypti* Rapid Index Survey (LiRAa) is the main method recommended by the National Dengue Control Program - PNCD to estimate the population density of *A. aegypti.* However, oviposition traps have been the target of research in several states of the Brazilian federation (BRASIL-PNCD, 2002; MS, 2013). This method demonstrates effectiveness in recording *Aedes* spp, thus corroborating the detection of vector infestation levels and egg positivity and density rates (POI and EDI) recorded in this research. With the larval survey and the positivity and egg density indices, it was possible to infer that *A. aegypti* has a great affinity for the urban perimeter (BARRETO E, et al., 2020; GONZÁLEZ OG, et al., 2021), which facilitates the spread of different arboviruses transmitted by the vector, becoming a significant concern for the spread of several arboviruses.

According to Costa AR, et al. (2016), the indices recorded in the ovitraps can be classified into parameters for monitoring mosquitoes of the genus *Aedes*: CONTROL, when the vector density registers POI values ≤ 40% and EDI ≤ 40 eggs, showing that the vector does not cause epidemic risks; ALERT, when the POI is between 41 to 60%, EDI of 41 to 60 eggs; and RISK, when the vector density of POI > 60%, EDI > 60 eggs, thus registering an increase in dengue cases. In Manaus/AM, the health districts under evaluation presented Building Infestation Indexes (IPO) of 16.6% and 66.6%, and Egg Density Index (IDO) of 15.2 and 72.55, oscillating between situations control and potential risk for the proliferation of A. aegypti.

It is noteworthy that the West district recorded the highest rates, (30 to 66.6%) with averages of 49.3% for IPO and 54.83% (29.4 to 72.5) for IDO, consolidating it as an area of risk. The IPO and IDO indices in the West district corroborate the results of the LIRAa carried out in the same collection period, where values greater than 4% indicate a clear risk of a dengue outbreak.

The values between the IPO and IDO indices and LIRAa reinforce the urgent need for effective interventions in this region, aiming to mitigate mosquito proliferation and, consequently, reduce the incidence of arboviruses. It is important to highlight that these indices are essential in A. aegypti control strategies, since the insertion of concise data on the types of containers found in homes allows for a more targeted approach, allowing adjustments and intensifications in control strategies, including awareness of the population with the elimination/protection of possible outbreaks.

According to the Ministry of Health, the LiRAa PI and BI are part of the control measures against *A. aegypti*, with the inclusion of data regarding the types of recipients, making it possible to redirect and/or intensify the control strategies adopted. Regarding the inspections carried out by LiRAa, Gama R, et al. (2007), in a comparative study between mosquiTRAP and the larval survey in the dry season in Minas Gerais, Brazil, found BI and PI indices of 0 to 1.7 throughout the collection period. In addition, in the present work, variations in BI and PI values were also founded. Although the species *A. aegypti* and *A. albopictus* are found in urban and semi-urban areas, according to Kamgang B, et al. (2017), *A. aegypti* presents anthropophilic behavior, with an adaptive preference for urban areas, with an emphasis on the interior of residences.

Thus, corroborating the records found in all health districts in Manaus - Amazonas. Although the number of specimens was not counted, we can infer that the species *A. aegypti* was predominant in the ovitraps installed. Thus, corroborating the records found in all health districts in Manaus/AM. The IPO and IDO values highlight the significant presence of the *Aedes* spp. mosquito in the monitored areas.

Although the precise number of specimens was not recorded, the analysis points to the predominance of the species *A. aegypti* in the ovitraps installed throughout the study. The high infestation of these vectors in different health districts highlights the importance of specific combat strategies in order to contain the spread of these mosquitoes and, consequently, reduce the incidence of diseases transmitted by them.

During the collection cycles, it was possible to record different rainfall levels, with the accumulation of water in the streets, the clogging of drains and, consequently, flooding was evident. As a result, household objects left in the open could be dragged into ravines and slopes, remaining there for an indefinite period of time, becoming potential breeding grounds for the development of mosquito vectors. According to Almeida RB e Aleixo NCR (2022), the Amazon region is characterized by having a humid and hot equatorial climate, which contributes to the proliferation of vectors even in periods of low precipitation (0.0 mm to 36 mm), a fact that which can be evidenced during the collection period (LI Y, et al., 2014; BARBOSA IR e SILVA LP, 2015; MELO RA, et al., 2021).

In the study conducted by Moraes BC, et al. (2019), when investigating the seasonality pattern in dengue notifications in Amazonian capitals, found a notable increase in cases during the rainy season, with significant peaks from January to March. This seasonal trend highlights the climatic influence on the dynamics of virus propagation, with the rainy season being a favorable factor for the proliferation of the mosquito vector. In contrast Oliveira LSB, et al. (2021) when studying the diversity of the mosquito *Aedes* spp. in Cuiabá, recorded infestation of specimens during the dry period.

This finding reinforces the adaptation capacity of *A. aegypti*, even in conditions less conducive to its reproduction. The results of this investigation converge with the egg density indices identified in the present research, highlighting that even during the dry period, in August and September, corresponding to the Amazonian summer, the presence of *A. aegypti* remains in the municipality. This highlights the importance of continuous control strategies, regardless of seasonal variations, and the need for preventive measures throughout the year must be strengthened. Acioli RV (2006), in a study on the use of oviposition traps, recorded the presence of *A. aegypti* in the peridomestic area of the neighborhoods of Recife-PE, a fact that proves the effectiveness of ovitraps even installed in the peridomestic area, that is, in places with less circulation of people.

Based on the results obtained in the present study, ovitraps are low-cost and highly sensitive traps, in addition to being easy to install, they are agile, as they are distributed in strategic locations throughout the home, which optimizes time, meaning there is no need to inspect possible foci of the *A. aegypti*. Unlike LiRAa, which actively searches possible breeding sites found in homes, and which may present difficulty in capturing larval larvae, as they tend to migrate to deeper parts of the breeding sites when disturbed or develop in difficult-to-access places such as pieces of glass arranged on walls, tree hollows and even at the bottom of tanks (FAY RW e ELIASON DA, 1966; BRAGA IA, 2020; OLIVEIRA SS, 2016).

CONCLUSION

In summary, both methods are necessary for monitoring areas infested by *A. aegypti*, in order to map which regions tend to suffer major impacts during endemic periods. In the four health districts of the city of Manaus/AM, monitoring with the aid of oviposition traps detected the presence of eggs in 38.8% of the installed ovitraps, while LIRAa detected the presence of the vector in 5.82% of the inspected residences. While ovitraps record the early detection of Aedes spp. mosquitoes through eggs in specific areas, LIRAa expands the analysis to identify the number of properties with the presence of containers with A. aegypti larvae, providing crucial data to guide control strategies and prevention.

Therefore, the integration of these methods offers a more robust and informed approach to the effective management of *A. aegypti* populations, contributing to the effectiveness of the measures adopted to combat diseases transmitted by this vector. The presence of *A. aegypti* identified in urban areas through the use of ovitraps and the rapid survey of the index for *A. aegypti* (LIRAa) signals the urgent need to program effective vector control strategies, since this vector has a preference for areas densely populated urban areas.

Therefore, ovitraps showed significant sensitivity in detecting eggs in the home region, being an excellent monitoring alternative and can be easily introduced into epidemiological surveillance actions since the deposited eggs can be collected and analyzed at regular intervals, providing a dynamic view of the presence of *A. aegypti* throughout the year, which can facilitate the identification of critical periods and the assessment of the effectiveness of control measures.

In short, the use of ovitraps offers a specific and sensitive approach, increasing robustness in data collection, as it detects specific areas within a municipality or neighborhood, added to the practicality of movement, low cost and easy installation. LIRAa differs methodologically from oviposition traps, as the active search is centered on capturing larvae, denoting a greater sampling effort when compared to capturing eggs, in addition to being carried out only three to four times a year.

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