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Exploring the breeding sites of *Aedes aegypti* (Diptera: Culicidae) in Camagüey, Cuba

Explorando los lugares de cría de Aedes aegypti (Diptera: Culicidae) en Camagüey, Cuba

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ABSTRACT

Mosquito-borne diseases continue to be of significant public health concern in various parts of the world, facilitated by the adaptations that certain species, such as *Aedes aegypti*, have managed to develop. With the aim of updating the bioecological knowledge of *Ae. aegypti* populations in a Health Area of Camagüey Province, Cuba, a comprehensive entomological survey was conducted both indoors and outdoors in 2018, covering the entire urban population, encompassing 36,584 households. *Aedes aegypti* colonized 52 types of breeding sites, of which 15 were found to be permanent and useful (29.0%), contributing 82.0% of the positivity. Ground-level tanks were particularly prominent (43.0%). Positivity in natural breeding sites remained low (0.11%). Larvae were collected in habitats with high levels of eutrophication (17.30%), grouped in 28 positive breeding sites, accounting for 3.32% of the total number of positive sites. No differences were observed in the total number of positive breeding sites and capture locations between climatic seasons, indicating a consistent pattern of positivity throughout the study year, during which six types of breeding sites exceeded a 70% monthly repetitiveness. These results underscore the importance of vigilance and control measures to be undertaken by residents in their respective households.

Keywords: *Aedes aegypti*, dengue, vector control, habitat, community engagement, Camagüey, Cuba.

RESUMEN

Las enfermedades transmitidas por mosquitos continúan siendo un importante problema de salud pública en diversas partes del mundo, facilitado por las adaptaciones que han logrado desarrollar algunas especies, como *Aedes aegypti*. Con el objetivo de actualizar el conocimiento bioecológico de las poblaciones de *Ae. aegypti* en un Área de Salud de la provincia de Camagüey, Cuba, se realizó

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una encuesta entomológica exhaustiva, tanto en interiores como en exteriores de las viviendas en 2018, abarcando a toda la población urbana, con un total de 36.584 hogares. *Aedes aegypti* colonizó 52 tipos de criaderos, de los cuales 15 resultaron ser permanentes y útiles (29.0 %), contribuyendo al 82.0 % de la positividad. Destacaron los tanques bajos (43.0 %). La positividad en los lugares naturales de cría fue baja (0.11 %). Se recogieron larvas en hábitats con altos niveles de eutrofización (17.30 %), agrupados en 28 sitios positivos, lo que representó el 3.32 % de la totalidad de los criaderos positivos. No se observaron diferencias en el número total de focos positivos y lugares de captura entre estaciones climáticas, lo que indica un patrón constante de positividad a lo largo del año de estudio, durante el cual seis tipos de criaderos superaron el 70 % de repetitividad mensual. Estos resultados acentúan la importancia de las medidas de vigilancia y control que deben adoptar los residentes en sus respectivos hogares.

Palabras clave: *Aedes aegypti*, dengue, control vectorial, hábitat, participación comunitaria, Camagüey, Cuba.

INTRODUCTION

Vector-borne diseases continue to hold significant public health relevance in various parts of the world, a trend that has been facilitated by the adaptations achieved by certain vectors, such as *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae). This situation has had implications for the emergence of increasingly intense and recurring epidemic outbreaks in several tropical countries, particularly in the Americas, with epidemics occurring cyclically every 3 to 5 years [1].

Currently, dengue is the arbovirus spreading most rapidly worldwide, with a significant increase that constitutes a serious global issue [2-4]. In the case of the Americas, the continent experienced the highest number of recorded dengue cases in 2023, with a total of 4,565,911 cases and 2,340 deaths [5]. The simultaneous circulation of other arboviruses, such as chikungunya and Zika, has complicated the situation in recent years, increasing the burden on healthcare services [6]. Consequently, there is a need to strengthen surveillance, triage, prompt diagnosis, and treatment of cases, as well as to intensify vector prevention and control measures. This underscores the ongoing need for the accumulation of bioecological evidence concerning mosquito species implicated in the transmission of these viruses to humans. Such evidence is essential for the development and implementation of integrated vector-focused strategies, emphasizing community-based surveillance and control measures [7-9].

Recent studies have demonstrated several determining factors that promote the dispersion of certain synanthropic species within the *Aedes* genus. Firstly, the wide range of breeding sites resulting from inadequate environmental hygiene practices [10,11] followed by the intensive and extensive use of chemicals (fertilizers and pesticides), indirectly affected various culicid species [12]. It is also important to consider the increase in human movements by sea, land, and air [13-15], as well as changes in climatic conditions. Rising temperatures and alterations in rainfall patterns have facilitated the introduction, establishment, and reproduction of invasive mosquitoes in distant locations from their original habitat [16].

The aim of the present study is to enhance the bioecological understanding of *Ae. aegypti* populations within a Health Area of Camagüey Province, Cuba. A broad comprehension of the preferred breeding sites of the immature stages of this mosquito species bears significant practical implications for the implementation of an efficient and context-specific vector control strategy.

MATERIAL AND METHODS

Study Area: The province of Camagüey is located approximately between 20°31'01"-22°29'00" N and 78°39'22"-76°57'00" W. The Health Area included in the study belongs to the municipality with the same name: 'Ignacio Agramonte' Health Area (IAHA), with a territorial extension of 4.5 km², and a total of 8,425 households.

Study Period: The entomological database from the Vector Control Department of IAHA was utilized for the period spanning January to December 2018. Two distinct climatic periods reported for Cuba were taken into consideration: the rainy season (May-October) and the dry season (November-April) [16].

Survey Technique: A 100% inspection of the urban universe, comprising 36,584 households, was conducted both indoors and outdoors. Each household received a monthly visit as part of Cuba's National Vector Control Network

planning. All types of deposits, including water storage recipients, small miscellaneous artificial deposits, drains, sewage pits and water registers, and natural breeding sites, were examined.

Sample Collection: In each container, the maximum number of larvae and/or pupae possible were collected using Pasteur pipettes and plastic trays, into which the water from each container had been previously poured. The samples were preserved in small jars with 70% alcohol. Primary data, including the address (highlighting the block), date, and type of container along with its specific location (indoor or outdoor), were recorded on the accompanying label.

Classification and Characterization of Containers: Following the criteria of Armada & Trigo [18], the containers were classified as permanent (P) and useful (U). This criterion was established based on the importance and priority that families assign to the various water-holding containers within households [19].

Taxonomic Identification: This was carried out at the Municipal Medical Entomology Laboratory of Camagüey, using the identification key provided by González Broche [20].

Data Processing: The type and total count of each positive container were quantified based on its location within each household during different climatic seasons, as well as its repetitiveness throughout the study period. The total number of positive PU containers for the vector, in terms of their significance, was compared between climatic seasons using a non-parametric chi-square test for 2-by-2 contingency tables with a significance level of $p \leq 0.05$.

Ethical Aspects: This research adhered to ethical standards to ensure the protection of personnel involved in field visits, as well as the laboratory technician responsible for the analysis and identification of the collected samples. This approach allowed the generation of new knowledge without violating established ethical principles in such cases. Furthermore, all authors involved in the research and the publication and dissemination of the results are responsible for the reliability and accuracy of the presented findings.

RESULTS

Fifty-two types of containers colonized by *Ae. aegypti* were characterized (figure 1), with 15 identified as PU (29.0%); despite constituting a minority, they contributed to 82.0% of the positivity. Among this variety of reservoirs, ground-level tanks stood out with 43.0% of larval samples of the species. Positivity in natural containers proved to be very low, at just 0.11%. Interestingly, it was observed that among the top ten types of containers, 80% had high utility for households. *Aedes aegypti* larvae were also collected in nine types of containers with high levels of eutrophication (17.30%): water register, ditch, latrine, downpipe, sewage pit, drainage, drain, pigsty floor and animal feeder, grouped in 28 positive containers, accounting for 3.32% of the total number of positive sites. The abundance of positive larval *Aedes* containers was significantly higher outdoors than indoors, attributed in part to the greater accessibility and number of containers for mosquito breeding outside households compared to indoors (table 1).

Table 1. Total number of positive deposits for *Aedes aegypti* in Camaguey in 2018, categorized by climatic season. Deposits identified as permanent and useful (PU) are highlighted.

Deposits	Total positive	Total positive rainy season	%	Total positive dry season	%	Total positive outdoors	Total positive outdoors rainy season	%	Total positive outdoors dry season	%
Ground-level tank	365	181	49.59	184	50.41	295	144	48.81	151	51.19
Cistern	109	55	50.46	54	49.54	84	41	48.81	43	51.19
Barrel	78	38	48.72	40	51.28	51	15	49.02	26	50.98
Bucket	38	14	36.84	24	63.16	23	9	37.50	15	62.50

Table 1. Total number of positive deposits for *Aedes aegypti* in Camaguey in 2018, categorized by climatic season. Deposits identified as permanent and useful (PU) are highlighted.

Deposits	Total positive	Total positive rainy season	%	Total positive dry season	%	Total positive outdoors	Total positive outdoors rainy season	%	Total positive outdoors dry season	%
Water basin	29	10	34.48	19	62.52	21	7	33.33	14	66.67
Elevated tank	23	18	78.26	5	21.74	20	16	80.00	4	20.00
Cooking pot	16	8	50.00	8	50.00	14	7	50.00	7	50.00
Used car tire	15	9	60.00	6	40.00	13	7	53.85	6	46.15
Animal drinker	14	5	35.71	9	64.29	13	4	30.77	9	69.23
Water register	13	4	30.77	9	69.23	10	3	30.00	7	70.00
Plastic vessel	13	7	53.85	4	46.15	7	3	42.86	4	57.14
Plant pot	11	9	81.82	2	18.18	10	8	80.00	2	20.00
Refrigerator tray	10	2	20.00	8	80.00	0	0	0.00	0	0.00
Spiritual vase	9	3	33.33	6	66.67	7	2	28.57	5	71.43
Can	9	8	88.89	1	11.11	8	7	87.50	1	12.50
Toilet	7	3	37.50	5	62.50	3	1	33.33	2	66.67
Tank lid	7	6		1	14.29	6	5	83.33	1	16.67
Plastic botlle	6	1	16.67	5	83.33	6	1	16.67	5	83.33
Milk can	6	2	33.33	3	66.67	5	2	40.00	3	60.00
Clay pot (tinajón)	5	1	20.00	4	80.00	5	1	20.00	4	80.00
Ditch	4	1	25.00	3	75.00	4	1	25.00	3	75.00
Puddle	4	1	25.00	3	75.00	3	1	33.33	2	66.37
Plastic bag	4	2	50.00	2	50.00	4	2	50.00	2	50.00
Latrine	3	3	100.00	0	0.00	2	2	100.00	0	0.00
Hole	3	1	33.33	2	66.67	2	1	50.00	1	50.00
Bowl	3	2	66.67	1	33.33	2	2	100.00	0	0.00
Downpipe	2	1	50.00	1	50.00	2	1	50.00	1	50.00
Sewage pit	2	0	0.00	2	100.00	2	0	0.00	2	100.00
Vessel	2	0	0.00	2	100.00	2	0	0.00	2	100.00
Kitchen sink	2	2	100.00	0	0.00	2	2	100.00	0	0.00
Scrap metal	2	2	100.00	0	0.00	2	2	100.00	0	0.00
Bathtub	2	1	50.00	1	50.00	2	1	50.00	1	50.00
Jar	2	2	100.00	0	0.00	2	2	100.00	0	0.00
Laundry sink	2	1	50.00	1	50.00	2	1	50.00	1	50.00
Bottle carrier	2	0	0.00	2	100.00	2	0	0.00	2	100.00
Cattle watering trough	2	0	0.00	2	100.00	2	0	0.00	2	100.00
Well	1	1	100.00	0	0.00	1	1	100.00	0	0.00
Flower vase	1	0	0,00	1	100.00	1	0	0.00	1	100.00
Car engine	1	0	0,00	1	100.00	1	0	0.00	1	100.00
Watering can	1	0	0,00	1	100.00	1	0	0.00	1	100.00
Coconut shell	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Drainage	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Hand wash basin	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Plastic canister	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Tree hole	1	1	100,00	0	0.00	1	1	100.00	0	0.00

Table 1. Total number of positive deposits for *Aedes aegypti* in Camaguey in 2018, categorized by climatic season. Deposits identified as permanent and useful (PU) are highlighted.

Deposits	Total positive	Total positive rainy season	%	Total positive dry season	%	Total positive outdoors	Total positive outdoors rainy season	%	Total positive outdoors dry season	%
Drain	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Glass bottle	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Pigsty floor	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Animal feeder	1	1	100,00	0	0.00	1	1	100.00	0	0.00
Tarpaulin	1	0	0,00	1	100.00	1	0	0.00	1	100.00
Shower curtain	1	0	0,00	0	0.00	0	0	0.00	0	0.00
Puddle in closet	1	0	0,00	0	0.00	1	0	0.00	0	0.00
TOTAL	841	413	49.11	428	50.89	653	322	49.31	331	50.69

Figure 1. Various water containers serving as breeding sites for *Aedes aegypti* in and around households in Camagüey in 2018. A) Ground-level tank; B) used tires; C) plant pot; D) tree hole; E) cistern; F) water register; G) spiritual vessels; H) barrels; I) toilet; J) cattle watering trough; and K) typical clay pot, commonly known in Cuba as 'tinajón'.

The positivity trend over months is illustrated in figure 2; however, no differences were observed in the total number of positive containers and capture locations between climatic seasons (table 2). On the other hand, table 3 presents the total number of months in which each type of container yielded positive results. Six containers, all of which were PU (100%), stand out, surpassing a 70% repeatability rate.

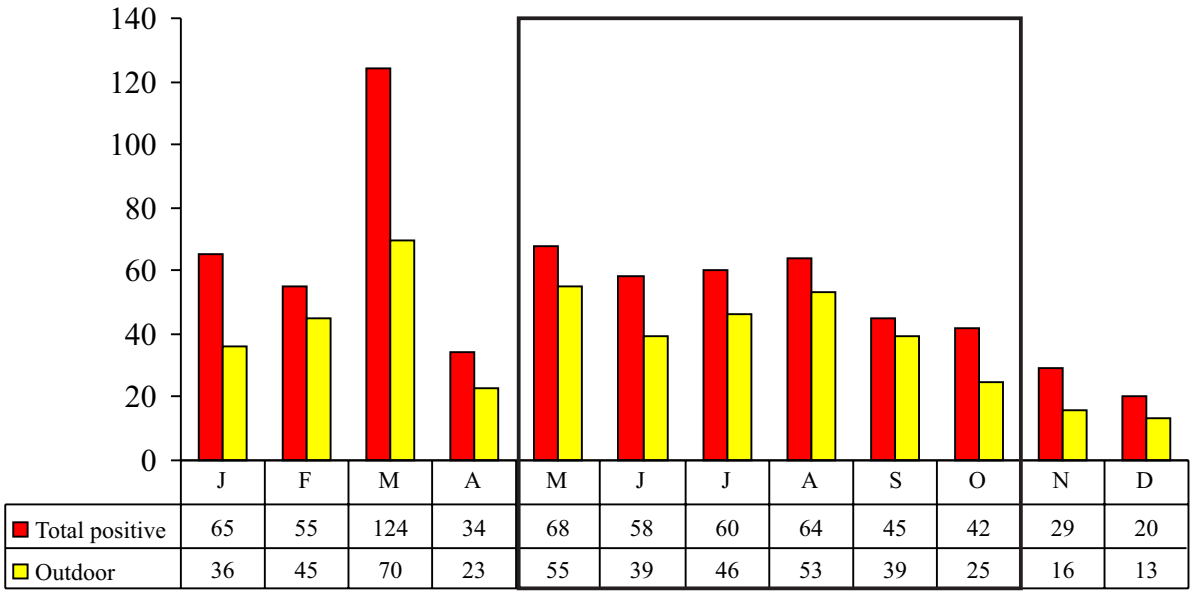

Figure 2. Monthly positivity of deposits with *Aedes aegypti* in Camagüey in 2018, by location in the household. Enclosed within the box are the months included in the rainy season (May-October).

Table 2. Positivity patterns of *Aedes aegypti* in Camaguey in 2018 across climatic seasons.

	Deposits according to climatic seasons			
	Dry season	Rainy season	Total	Significance level
Positives PU	358	335	693	$p > 0.05$
Negatives PU	70	78	148	
Total	**428**	**413**	**841**	
Outdoor positives PU	331	322	653	$p > 0.05$
Indoor positives PU	27	13	40	
Total	**358**	**335**	**693**	

Table 3. Repetitiveness pattern of positive deposits for *Aedes aegypti* in Camaguey in 2018. The 10 most abundant types are highlighted in grey, with those permanent and useful (PU) in bold.

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Deposits	**Times repeated in the year**	**Times repeated in rainy season**	**Times repeated dry season**
Ground-level tank	12	6	6
Cistern	12	6	6
Bucket	12	6	6
Barrel	11	5	6
Water basin	11	5	6

Table 3. Repetitiveness pattern of positive deposits for *Aedes aegypti* in Camaguey in 2018. The 10 most abundant types are highlighted in grey, with those permanent and useful (PU) in bold.

Deposits	Times repeated in the year	Times repeated in rainy season	Times repeated dry season
Animal drinker	9	4	5
Cooking pot	8	4	4
Used car tire	8	4	4
Water register	7	2	5
Toilet	7	3	4
Elevated tank	5	3	2
Plant pot	5	4	1
Refrigerator tray	5	2	3
Clay pot (tinajón)	4	1	3
Plastic vessel	4	3	1
Can	4	3	1
Tank lid	4	1	3
Milk can	4	2	2
Puddle	4	1	3
Spiritual vase	3	2	1
Ditch	3	1	2
Plastic bag	3	1	2
Hole	3	2	1
Bowl	3	2	1
Latrine	2	2	0
Downpipe	2	1	1
Sewage pit	2	0	2
Plastic botlle	2	1	1
Kitchen sink	2	2	0
Jar	2	2	0
Laundry sink	2	1	1
Well	1	1	0
Flower vase	1	0	1
Vessel	1	0	1
Scrap metal	1	1	0
Bathtub	1	1	0
Bottle carrier	1	0	1
Cattle watering trough	1	0	1
Car engine	1	0	1
Watering can	1	0	1
Coconut shell	1	1	0
Drainage	1	1	0
Hand wash basin	1	1	0
Plastic canister	1	1	0
Tree hole	1	1	0
Drain	1	1	0
Glass bottle	1	1	0
Pigsty floor	1	1	0
Animal feeder	1	1	0
Tarpaulin	1	0	1
Shower curtain	1	0	1
Puddle in closet	1	0	1
Total	**186**	**94**	**92**

DISCUSSION

Given the current emergence and re-emergence of mosquito-borne diseases, new challenges arise in the design and implementation of vector control programmes. Dengue, as well as other emerging arboviruses of significant importance, is a major global economic burden, severely affecting the Caribbean region [22,23]. In Cuba, there was transmission of dengue in eight provinces in 2023. However, the island has remained free of Zika and chikungunya since 2017 [24]. Integrated vector management under the 'One Health' approach requires greater community and cross-sectoral involvement. Encouraging families to give proper attention to containers identified as 'priority' is crucial for reducing the adverse effects associated with *Aedes*-borne diseases.

In this regard, there is a pressing need to increase the number of entomological studies conducted in situ to understand the behavior of *Ae. aegypti* in specific localities. Historically, Cuba has been the insular Caribbean country with the most research conducted in the field of medical and veterinary entomology, with a strong focus on mosquito studies for several decades [25]. These research efforts enable the development and implementation of a more accurate and locally tailored strategic design, focusing on the most productive containers, as they significantly contribute to outbreaks and epidemics [26].

Considering the obtained results, the presence of the vector is observed in a wide variety of breeding sites, indicating that the community-level actions taken have proven to be insufficient. This allows the mosquito to successfully adapt to environments subjected to strong disturbances, such as the urban setting of Camagüey. Furthermore, the breeding sites with the highest positivity remains consistent with previous studies, with cisterns and barrels standing out, and ground-level tanks being identified as the 'key container' in Cuba [19,27-29].

Moreover, the presence of *Ae. aegypti* in eutrophicated water containers underscores the intriguing strategic adaptations that the species is undergoing, a phenomenon previously highlighted by Diéguez et al. [8], and Marquetti et al. [28]. Consequently, the species is expanding its spatial niche to encompass more polluted habitats [29]. This, combined with the absence of differences in abundance levels between climatic seasons (with nearly uniform behavior throughout the year), confirms the adaptive abilities characteristic of 'r' strategists that the species is acquiring [30]. Additionally, this vector can coexist with other synanthropic aedine species, such as *Aedes albopictus* (Skuse, 1894) or *Aedes vittatus* (Bigot, 1861), which have recently been introduced to the continent and Cuba [31,32]. These species also pose a threat to public health, each having different bioecological requirements different from those of *A. aegypti*.

In previous studies conducted in Camagüey, a higher presence of vector-positive breeding sites was observed outdoors, where families are less active in revising and protecting potential deposits that could be colonized by the vector [33], which partly aligns with our results. Therefore, given the reported infestation level, we recommend prioritizing prevention and control measures that strengthen environmental management actions, promote the implementation of physical and biological methods for breeding site control, accurately delineate high-risk transmission areas (risk stratification), and allocate resources to areas with significant population concentrations. Additionally, it is crucial to enhance quality control measures for field workers, ensuring adherence to personal protective measures during both focal treatment and adulticidal treatment (fumigation).

In Cuba, various forms of household treatment are routinely conducted, including the application of granulated Temefos and the inspection of potential water-holding containers. Additionally, chemical interventions with pyrethroids are implemented in areas with high adult infestation levels. Therefore, it is crucial to enhance community engagement in the self-inspection of residential or workplace premises, a practice known in Cuba as 'autofocal familiar' and 'autofocal laboral', respectively, which should occur weekly [34]. It is worth noting that proper execution of these activities could prevent the majority of positive breeding sites.

In summary, *Ae. aegypti* exhibits a wide range of breeding sites, with a preference for domestic water containers, particularly ground-level tanks, cisterns, and barrels. The adoption of the 'self-inspection' approach can greatly aid in reducing breeding sites in the study area, highlighting its significance in the surveillance and control efforts to be carried out by residents in their respective households.

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