



Research article

Field investigation of *Aedes* vectors during dengue outbreak in Araceli, Palawan, the Philippines

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Abstract

Importance of work: In mid-2023, the municipality of Araceli in Palawan, the Philippines, experienced a major dengue outbreak, prompting an urgent field entomological investigation.

Objective: To determine the density, distribution and breeding habitats of *Aedes* mosquito vectors in the two most affected barangays (the smallest administrative divisions in the Philippines) of Poblacion and Dagman to inform evidence-based control strategies.

Materials and Methods: In total, 279 structures were surveyed, followed by the collection of both immature and adult mosquitoes in the two barangays in Araceli. These samples were identified morphologically per container and per house. In addition, all female *Aedes* vectors collected were tested for dengue virus detection based on real-time polymerase chain reaction (PCR).

Results: In total, 3,439 mosquito specimens were collected, comprising larvae (74.41%), pupae (12.88%) and adults (12.71%). *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) were the predominant species identified. Key breeding containers were mainly outdoor artificial water storage items such as pails, drums and discarded items. The entomological indices—House Index (HI), Container Index (CI), Breteau Index (BI), Pupal Index (PI), Pupae per Person Index (PPI) and Adult House Index (AHI)—all exceeded national and World Health Organization threshold values, confirming high dengue transmission risk. Two-sample proportion tests revealed no significant differences in larval indices between households with and without dengue history (all $p > 0.05$); however, lower AHI ($p = 0.041$) and PPI ($p = 0.006$) values in the case-history households suggested post-intervention suppression.

Main findings: Although no dengue virus was detected in adult mosquitoes based on PCR analysis, the sustained presence of vectors suggested ongoing transmission potential. These findings underscore the importance of integrating larval, pupal and adult surveillance, insecticide resistance monitoring and community engagement into local vector control programs.

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Introduction

Dengue remains the most important mosquito-borne viral disease in the Philippines (Espino et al., 2013). Between 2010 and 2017, the country experienced a steady rise in dengue cases, partly attributed to the limited success of existing vector control measures (Department of Health, 2018a). In 2019, the Philippines reported 437,563 cases, prompting the declaration of a National Dengue Epidemic (Ong, et al., 2022). More recently, in 2023, the World Health Organization identified the Philippines as the most affected country in the Western Pacific Region, with 167,355 reported cases and 575 deaths (case fatality rate [CFR] = 0.34%), followed by Vietnam with 149,557 cases and 36 deaths (CFR = 0.02%) (World Health Organization, 2023). While other dengue-endemic countries have adopted innovative vector control strategies, such as the sterile insect technique and Wolbachia-based biocontrol, the Philippines still relies heavily on chemical-based interventions, particularly during outbreaks. Although non-insecticidal methods are prioritized where feasible, the strategic use of insecticides remains an essential component for rapidly interrupting transmission (Sparks and Nauen, 2015; World Health Organization, 2016, 2017; Insecticide Resistance Action Committee, 2019). In line with this, the Department of Health, through the National *Aedes*-borne Viral Diseases Prevention and Control Program, issued Administrative Order 2018-0021 to guide the outbreak response using the enhanced 4S Strategy (search and destroy, seek early consultation, self-protection measures, say yes to fogging only during outbreaks) along with other measures such as the use of insecticide-treated screens, school-based long-lasting insecticidal nets, targeted indoor and outdoor residual spraying and using both larvicides and adulticides (Department of Health, 2018b).

However, extensive and repeated use of insecticides across agricultural, domestic and public health sectors has contributed to the development of insecticide resistance in vector populations (Garcia et al., 2018; Sternberg and Thomas, 2018). This resistance arises from the selection pressure induced by similar active ingredients used in crop protection and household pest control (Aktar et al., 2009; Insecticide Resistance Action Committee, 2007, 2019). When resistance develops, mosquito populations may survive standard doses of insecticides or avoid contact with treated surfaces altogether, compromising vector control effectiveness.

According to the Department of Health Manual of Procedures (Department of Health, 2018b), vector surveillance

is necessary under various circumstances: during suspected resistance, pre- and post-intervention, in high- or low-risk zones and during outbreaks. In mid-2023, the Municipal Health Office of Araceli and the Provincial Health Office of Palawan reported a rapid increase in dengue cases—from only 8 cases in morbidity week 18 (March 26–31) to 308 cumulative cases and 5 deaths—substantially exceeding the five-year annual average of 43 cases, despite ongoing campaigns and interventions following Department of Health guidelines (Department of Health, 2018b, 2018c; Provincial Health Office–Palawan, 2023).

To determine the entomological factors underlying this surge, an investigation was carried out using both traditional and expanded indices: the House Index (HI), Container Index (CI), Breteau Index (BI), Pupal Index (PI), Pupae per Person Index (PPI), Adult House Index (AHI) and identification of key breeding containers. These methods followed World Health Organization protocols (World Health Organization, 1995, 2003, 2009, 2011) and national guidelines (Department of Health, 2018b). Specifically, the study aims were: (1) to determine the presence, density and distribution of *Aedes* vectors in Araceli, Palawan; (2) to assess infestation levels using standardized indices; and (3) to identify key breeding containers to guide evidence-based vector control measures.

Materials and Methods

Ethical considerations

This entomological survey was conducted as part of the official public health response to a reported dengue outbreak in the municipality of Araceli, Palawan, the Philippines. The study involved only the collection of mosquito specimens for vector surveillance and did not include any human participation or collection of human biological samples. As such, the activities were considered exempt from formal ethics review based on national and institutional guidelines governing non-invasive public health surveillance. All field activities were carried out in close coordination with the Department of Health, the Provincial Health Office of Palawan, the Municipal Health Office of Araceli and Barangay Health Volunteers in the affected communities of Poblacion and Dagman. The conduct of the study adhered to relevant ethical standards and established procedures for entomological surveillance.

Study sites

Entomological collections were conducted 2–3 July 2023 in the municipality of Araceli, located in northeastern Palawan (10.3333° N, 119.6000° E), in response to a substantial increase in reported dengue cases from May to June 2023 that exceeded both the alert and epidemic thresholds (Provincial Health Office, 2023). Araceli shares its southern boundary with the municipality of Dumarang and comprises 15 barangays (the smallest administrative divisions in the Philippines). The investigation focused on the two barangays with the highest dengue case burden (Poblacion and Dagman), accounting for 64.46% and 11.01% of total reported cases in the municipality, respectively (Provincial Health Office, 2023). Barangay Poblacion serves as the urban and administrative center of Araceli. It is the most densely populated and economically active barangay, housing key government offices, schools, commercial establishments and the municipal seaport. Residences in this area are closely packed, with minimal vegetation observed around the surveyed households. In contrast, Barangay Dagman is a rural community situated to the south of Poblacion. It is accessible via a short boat ride (15 min) or a longer land journey (1–1.5-hr) from the town center. Residential structures in Dagman are spaced farther apart and the area is characterized by extensive vegetation, including trees and shaded surroundings near homes. Systematic inspections of both indoor and outdoor premises were conducted in these two barangays as a part of the entomological surveillance activities.

Entomological survey

A field entomological survey was conducted in Poblacion and Dagman during 2–3 July 2023, in response to the sudden increase in dengue cases in the municipality of Araceli. In Poblacion, a total of 171 houses, 2 schools and 1 church were surveyed, while in Dagman, 104 houses and 1 school were included (total 279 structures). Survey coverage was guided by reported dengue cases at the time of investigation, with 27 confirmed cases (including three deaths) in Poblacion and 21 cases in Dagman (Provincial Health Office, 2023), as shown in Fig. 1. These case clusters served as the basis for identifying target areas for entomological collection. The study utilized both immature and adult mosquito collection techniques. With the assistance of local health workers, recent dengue case households were identified and designated as index houses. Using a buffer zone (200 m) around each index house, surrounding households were selected for entomological surveillance, aligning with World Health Organization (2003, 2009) and national guidelines (Department of Health, 2018b). Spatial mapping of case distribution and collection coverage was conducted by trained entomologists and georeferenced using handheld GPS devices (eTrex® 10; Garmin) and visualized in the QGIS software (version 3.44.2; QGIS Development Team, 2025), as shown in Fig. 1.

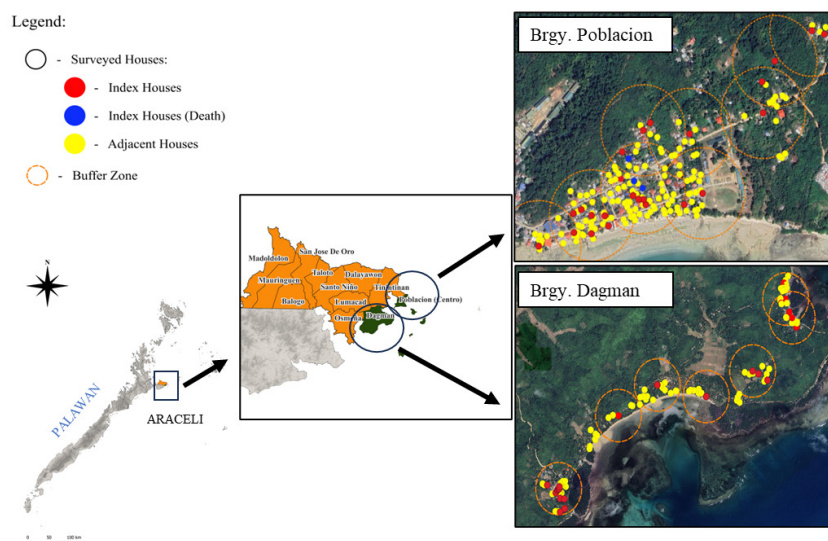


Fig. 1 Map of entomological outbreak investigation sites in Barangay (Brgy.) Poblacion and Brgy. Dagman, Araceli, Palawan, the Philippines, showing houses surveyed with history of dengue (red dots) and reported deaths (blue dots) and adjacent houses that were negative for dengue (yellow dots) up to 200 m radius (circular dotted orange line)

The investigation team was composed of personnel from the Research Institute for Tropical Medicine (RITM), the Provincial Health Office of Palawan, Provincial Department of Health Office in Palawan and Barangay Health Volunteers. Prior to field deployment, team members received standardized training and orientation. Four survey teams were formed, each with 4–5 members. The team roles included a household interviewer (responsible for collecting household information and completing survey forms), collectors of immature mosquitoes (tasked with inspecting and collecting larvae and pupae from water-holding containers), a collector of adult mosquitoes (conducted based on adult mosquito sweep-netting) and a GPS recorder (who documented the coordinates of each collection site). Barangay Health Volunteers assisted in household access and initial container identification, while RITM-trained collectors performed the sampling. Standardization was based on pre-field training (such as pipette and dipper protocols) and by RITM staff oversight of each team, minimizing bias through duplicate checks (in 10% of containers) and checking for GPS-logged consistency.

Adult mosquitoes were collected indoors using sweep nets, targeting resting areas such as dark corners, behind furniture (beds, chairs, tables) and on hanging clothes. Sweep netting was conducted in a figure-eight motion across identified locations. Homeowners were invited respectfully to accompany the survey team during indoor inspections to ensure transparency. Collected mosquitoes were aspirated using suction tubes, stored in labeled plastic bags and transported for further identification and analysis.

During each inspection, all indoor and outdoor water-holding containers were examined for the presence of immature *Aedes* mosquitoes. For small and narrow containers, pipettes and dippers were used to extract larvae and pupae, while a five-sweep netting method was applied to larger containers such as drums. Positive samples were placed in labeled plastic bags indicating container type, location (indoor or outdoor), household number and collector's name. Containers with no captured sample were recorded to ensure a complete survey profile. Samples were sealed with sufficient air and stored upright in thermo-chests to maintain appropriate temperature and conditions during transport to the field processing station.

This integrated approach allowed for the comprehensive collection of immature and adult stages, enabling the calculation of both traditional and expanded entomological indices.

Mosquito identification and molecular testing

All collected mosquito specimens—larvae, pupae and adults—were processed and identified using standard entomological procedures. Larvae and pupae were transferred carefully to Petri dishes or glass slides using a plastic pipette and examined individually under a stereomicroscope. Identification of larvae focused on the presence and arrangement of comb scales on the lateral side of the 8th abdominal segment and thoracic spines, as diagnostics for distinguishing *Aedes aegypti* and *Aedes albopictus* in the Philippines (Rueda, 2004). Pupae were differentiated based on paddle margins, setae placement on the terminal abdominal segment and genital lobe morphology for sex identification (Fig. 2; Carvalho et al., 2014). Adult mosquitoes were immobilized by freezing and identified using validated taxonomic keys: *Aedes* vectors via Rueda (2004); and other genera via Rattanarithikul et al. (2005a, 2005b, 2006, 2007, 2010). Distinguishing features of male and female *Ae. aegypti* and *Ae. albopictus* pupae (dorsal view) based on genital lobes are shown in Fig. 2.

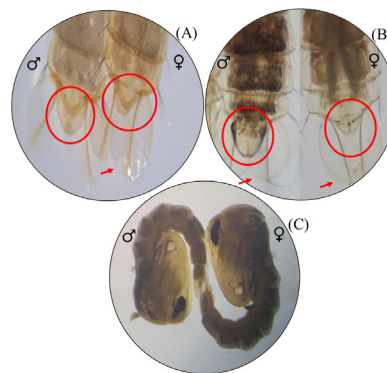


Fig. 2 Distinguishing between male and female pupae based on the genital lobe, following Carvalho et al. (2014): (A) *Aedes aegypti*, dorsal view; (B) *Aedes albopictus*, dorsal view; (C) lateral view of *Aedes* male and female pupae. Red circles indicated the genital lobe, located at the end of the pupal abdominal segments just below the paddles. At the pupal stage, the two dengue vectors can also be distinguished by the paddles margins (red arrow); with *Ae. albopictus* paddles are hairy compared with those of *Ae. Aegypti*. In addition, (C) males (♂) are generally smaller than females (♀).

For molecular analysis, female *Aedes* vectors from the same household were pooled (up to $n = 10$ per pool) in pre-labeled cryovials with silica gel and filter paper (labeling: species, household, date, code, location). Samples were shipped to the Department of Medical Entomology, RITM, Muntinlupa City, the Philippines for dengue virus detection based on real-time polymerase chain reaction (RT-PCR). Viral RNA extraction used the QIAamp Viral RNA Mini Kit (Qiagen; Hilden, Germany),

modified per manufacturer guidelines: pools were homogenized in 250 μ L phosphate-buffered saline with sterile micropestles, yielding 60 μ L eluates; the extracts were stored at -40°C until use. Approximately 50 pools from 94 female *Ae. aegypti* were tested using two complementary one-step multiplex RT-PCR assays. Protocol 1 (LRD-DME-FM-006) targeted DEN-1–4 with serotype-specific primers/probes (Johnson et al., 2005): DEN-1 (F: 5'-CAAAAGGAAGTCGTGCAATA-3', R: 5'-CTGAGTGAATTCTCTCTACTGAACC-3', Probe: FAM-CATGTGGTTGGGAGCACGC-BHQ-1); DEN-2 (F: 5'-CAGGTTATGGCACTGTCACGAT-3', R: 5'-CCATCTGCAGCAACACCATCTC-3', Probe: HEX-CTCTCCGAGAACAGGCCTCGACTTCAA-BHQ-1); DEN-3 (F: 5'-GGACTGGACACACGCACTCA-3', R: 5'-CATGTCTCTACCTTCTCGACTTGTCT-3', Probe: TR-ACCTGGATGTCGGCTGAAGGAGCTTG-BHQ-2); and DEN-4 (F: 5'-TTGTCTTAATGATGCTGGTCG-3', R: 5'-TCCACCTGAGACTCCTTCCA-3', Probe: CY5-TTCTACTCCTACGCATCGCATTCG-BHQ-3) (Johnson et al., 2005). Each 25 μ L reaction contained nuclease-free water, 1 μ M primer, 0.18 μ M probe, 1X SuperScript™ III RT/Platinum™ Taq Mix (Invitrogen; Massachusetts, USA) and 5 μ L sample. The cycling regime was reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 2 min, then 45 cycles at 95°C for 15 s and 60°C for 1 min using the on Bio-Rad CFX96 Deep Well RT-PCR Touch (Bio-Rad Laboratories; California, USA). Sampling yield a C_q value ≤ 36 were considered positive. Protocol 2 (LRD-DME-WI-035) used the genesig® Dengue/Zika/Chikungunya Multiplex Kit (PrimerDesign, London, UK) per instructions: 4 μ L nuclease-free water, 10 μ L OneStep Master Mix (PrimerDesign; London, UK), 1 μ L primer/probe mix and 5 μ L sample. The cycling regime was: 55°C for 10 min, 95°C for 2 min, then 50 cycles at 95°C for 10 s and 60°C for 60 s on Bio-Rad CFX96 (Bio-Rad Laboratories; California, USA). Positive (DENV-spiked) and no-template controls validated sensitivity and specificity.

Entomological indices

Entomological indices were calculated following standard guidelines using Equations 1–6 (World Health Organization, 2003, 2011):

$$\text{HI} = (\text{Number of houses positive for } Aedes \text{ larvae \& pupae}) / (\text{Total houses inspected}) \times 100 \quad (1)$$

$$\text{CI} = (\text{Number of positive containers for } Aedes \text{ larvae \& pupae}) / (\text{Total houses inspected}) \times 100 \quad (2)$$

$$\text{BI} = (\text{Number of positive containers for } Aedes \text{ larvae \& pupae}) / (100 \text{ houses inspected}) \quad (3)$$

$$\text{PI} = (\text{Number of } Aedes \text{ pupae}) / (\text{Total houses inspected}) \times 100 \quad (4)$$

$$\text{PPI} = (\text{Number of } Aedes \text{ pupae}) / (\text{Total houses inspected}) \times 100 \quad (5)$$

$$\text{AHI} = \text{Number of houses positive for adult mosquitoes} / (\text{Total houses inspected}) \quad (6)$$

Thresholds for high transmission risk were $\text{HI} > 4\%$, $\text{CI} > 4\%$ and $\text{BI} > 20$ (World Health Organization, 2003, 2011). Households were stratified based on dengue case history (case versus non-case).

Statistical analysis

Two-sample proportion z tests were used to compare larval (HI, CI, BI), pupal (PI, PPI) and adult (AHI) indices between groups. Statistical significance was set at $p < 0.05$. All analyses were conducted in R (version 4.3.1; R Core Team, 2023).

Results

In total, 3,439 mosquito specimens were collected the across 279 structures and 2,007 water-holding containers (Table 1). Immature stages comprised 87.29% (larvae 74.41% [$n = 2,559$], pupae 12.88% [$n = 443$]) and adults 12.71% ($n = 437$). *Aedes aegypti* was predominant (64.43%, $n = 2,215$), followed by *Aedes albopictus* (25.04%, $n = 861$); combined, these vectors accounted for 89.5% of specimens. Other species included *Culex quinquefasciatus* (8.96%, $n = 308$, filariasis vector) and minor *Aedes* and non-*Aedes* taxa. *Aedes* pupae totaled 407 (*Ae. aegypti* 309, *Ae. albopictus* 98). In Poblacion ($n = 1,669$ specimens), *Ae. aegypti* dominated (72.56%), whereas in Dagman ($n = 1,770$), *Ae. albopictus* was more prevalent (31.58% versus *Ae. aegypti* 56.72%).

Of the 2,007 containers inspected, 146 (7.28%) were positive for immature mosquito forms (Table 2). Outdoor containers predominated (83.2% of positives), with water storage items as key sites: pails (25.3% of positives, $n = 36$), drums (27.4%, $n = 39$) and jugs (6.8%, $n = 10$). No single type exceeded 55% of pupae productivity (Fig. 3); however, the aggregated storage containers (pails, drums, jugs) accounted for 68.5% of positives. Indoor positives were low (4.3%, $n = 28$).

Table 1 Summary of immature (larvae and pupae) and adult mosquito species collected and identified from Barangay (Brgy.) Poblacion and Brgy. Dagman, Araceli, Palawan, July 2–3, 2023

Mosquito species	Brgy. Poblacion						Brgy. Dagman				Total
	Larvae	Pupae ♀	Pupae ♂	Adult ♀	Adult ♂	Larvae	Pupae ♀	Pupae ♂	Adult ♀	Adult ♂	
<i>Aedes aegypti</i> ^a	894	82	105	72	57	847	74	48	22	14	2,215
<i>Aedes albopictus</i> ^a	262	28	12	0	0	501	21	37	0	0	861
<i>Aedes flavipennis</i>	0	0	0	0	0	5	0	0	0	0	5
<i>Aedes malayensis</i>	0	0	0	0	0	0	9	7	0	0	16
<i>Aedes vexans</i>	0	0	0	1	0	0	0	0	0	0	1
<i>Armigeres subalbatus</i>	0	10	1	0	0	0	6	3	2	0	22
<i>Culex quinquefasciatus</i> ^b	28	0	0	69	45	11	0	0	98	57	308
<i>Culex</i> sp.	1	0	0	0	0	0	0	0	0	0	1
<i>Toxorhynchites</i> sp.	2	0	0	0	0	7	0	0	0	0	9
<i>Uranotaenia</i> sp.	0	0	0	0	0	1	0	0	0	0	1
Total	1,187	120	118	142	102	1,372	110	95	122	71	3,439

♀ = female, ♂ = male; ^a = vectors of dengue, Zika and chikungunya; ^b = vector of filariasis.

Table 2 Summary of containers found and inspected for *Aedes* vectors in Barangay (Brgy.) Poblacion and Brgy. Dagman, Araceli, Palawan, July 2–3, 2023

Type of Container	Brgy. Poblacion			Brgy. Dagman			Total
	Indoor (+/–)	Outdoor (+/–)	Total	Indoor (+/–)	Outdoor (+/–)	Total	
Jug	2/271	4/163	440	0/60	4/28	92	532
Pail	4/141	9/106	260	5/45	18/73	141	401
Drum	3/65	16/78	162	5/91	15/94	205	367
Basin	0/22	6/70	98	2/9	12/113	136	234
Jar	0/63	0/2	65	0/83	3/57	143	208
Bottle	0/23	5/23	51	0/26	1/51	78	129
Flower vase	2/8	0/0	10	1/0	0/0	1	11
Tire	0/0	7/3	10	0/0	3/3	6	16
Discarded material	0/0	4/3	7	0/0	4/6	10	17
Bamboo fence	0/0	0/5	5	0/0	1/9	10	15
Clam shell	0/0	0/4	4	0/0	0/0	0	4
Dish drain	2/1	0/1	4	1/2	0/0	3	7
Plant axil	0/0	0/4	4	0/0	0/1	1	5
Flower pot	0/0	0/3	3	0/0	0/0	0	3
Coconut shell	0/0	0/2	2	0/0	0/5	5	7
Feeding bowl	0/0	0/2	2	0/0	0/0	0	2
Concrete water filter	0/0	1/0	1	0/0	0/0	0	1
Cup	0/0	0/1	1	0/0	0/5	5	6
Deep well	0/0	0/1	1	0/0	0/0	0	1
Flooded floor	0/0	0/1	1	0/0	0/0	0	1
Pitcher	0/0	1/0	1	0/0	0/0	0	1
Washing machine	0/0	1/0	1	1/0	0/0	1	2
Bamboo stump	0/0	0/0	0	0/0	3/0	3	3
Boots	0/0	0/0	0	0/0	0/2	2	2
Coconut leaflets	0/0	0/0	0	0/0	0/1	1	1
Frying pan	0/0	0/0	0	0/0	0/1	1	1
Pan	0/0	0/0	0	0/0	0/2	2	2
Pot	0/0	0/0	0	0/0	0/2	2	2
Shell	0/0	0/0	0	0/0	0/3	3	3
Shoe rack	0/0	0/0	0	0/0	0/1	1	1
Thermo-chest	0/0	0/0	0	0/0	0/1	1	1
Tin can	0/0	0/0	0	0/0	0/20	20	20
Toilet bowl	0/0	0/0	0	0/0	0/1	1	1
Grand total	13/594	54/472	1,133	15/316	64/479	874	2,007

+ = *Aedes* vector found; – = *Aedes* vector not found.

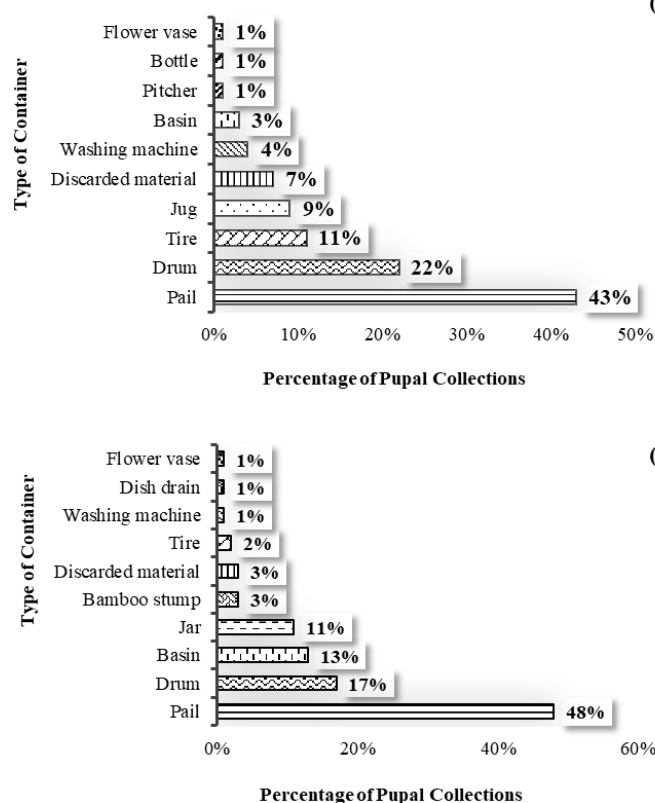


Fig. 3 Percentages of water-holding containers at study sites positive for *Aedes* pupae in: (A) Barangay Poblacion; (B) Barangay Dagman

Entomological indices exceeded the alert thresholds (World Health Organization, 2003, 2011), as shown in Table 3. In addition, pooled HI = 31.8%, CI = 7.3% and BI = 53.3, which were all greater than the National Department of Health risk levels of HI $\geq 5\%$, CI $\geq 5\%$, BI ≥ 20 (Department of Health, 2018c). Expanded indices indicated high emergence potential, with pooled PI = 148.6 (*Ae. aegypti/albopictus* pupae per 100 houses), PPI = 32.4 (per 100 persons, assuming 1,295 surveyed residents). Furthermore,

Table 3 Entomological indices in Barangay (Brgy.) Poblacion and Dagman, Araceli, Palawan, July 2-3, 2023 (corrected per World Health Organization standards)

Index	Brgy. Poblacion	Brgy. Dagman	Pooled
House index (%)	27.65	38.46	31.8
Container index (%)	5.91	9.04	7.3
Breteau index	39.41	76.0	53.3
Pupal index ^a	133.5	173.1	148.6
Pupae per person index ^b	24.64	48.09	32.4
Adult house index (%)	42.35	21.15	34.7

^a = *Aedes (aegypti + albopictus)* pupae per 100 houses; ^b = per 100 persons (surveyed population: Poblacion 921, Dagman 374, pooled 1,295).

(A) AHI = 34.7% reflected substantial adult infestation. Urban Poblacion has a higher AHI (42.35%) than rural Dagman (21.15%); however, the PPI was higher in Dagman (48.09 versus 24.64), suggesting rural productivity risks.

Comparisons by case history (48 case households versus 227 non-case; 1,203 versus 804 specimens) showed no differences in larval indices (HI, $p = 0.465$; CI, $p = 0.312$; BI, $p = 0.221$) (Table 4). However, case households had lower AHI ($p = 0.041$) value, consistent with recent interventions. Molecular testing of 50 pools (94 *Ae. aegypti* females) detected no DENV (all $C_q > 36$).

Table 4 Comparison of *Aedes* indices between households with and without a history of dengue in Araceli, Palawan, the Philippines

<i>Aedes</i> indices	With the dengue history	Without the dengue history	Difference	p-value
House index	27.08	32.47	-5.38	0.4651
Container index	7.47	7.23	0.24	0.8738
Breteau index	58.33	51.08	7.25	0.3601
Adult house index	18.75	33.77	-15.02	0.041

Discussion

The entomological investigation in Araceli, Palawan revealed intense *Aedes* vector infestation during the 2023 dengue outbreak, with all indices exceeding the thresholds of World Health Organization (2003, 2011) thresholds, thereby confirming elevated transmission risk. The high densities of *Aedes aegypti* and *Aedes albopictus*—coupled with the predominance of outdoor breeding in water storage containers—underscored persistent environmental and behavioral drivers of dengue transmission in both the urban and rural settings. The dominance of *Ae. aegypti* in the densely populated Poblacion Barangay reflected its strong domestic adaptation, while the substantial presence of *Ae. albopictus* in the vegetated Dagman Barangay highlighted the risk of peri-domestic transmission, consistent with regional observations across Southeast Asia (World Health Organization, 2011).

Traditional larval indices (HI 31.8%, CI 7.3%, BI 53.3%) all surpassed the thresholds of the World Health Organization (2003, 2011) and the alert thresholds of the Department of Health (2018c) of HI $> 4\%$, CI $> 4\%$ and BI > 20 . Such values aligned with epidemic conditions reported in prior outbreaks in the Philippines, where BI > 50 often signaled heightened dengue transmission (Department of Health, 2018b). Expanded indices, such as PI (148.6) and PPI (32.4), revealed high

adult emergence potential, supporting other reported findings that pupal-based measures provided a superior correlation with dengue incidence (Focks, 2003; Chang and Hii, 2011). The AHI (34.7%) confirmed substantial indoor vector presence, emphasizing the importance of complementing larval surveillance with adult monitoring in outbreak investigations (World Health Organization, 2016). Furthermore, this finding merits the use of targeted indoor residual spraying with known effective compounds.

The predominance of breeding in specific outdoor water storage items (pails, drums, jugs) accounted for nearly 70% of positive containers, reinforcing the urgent need for source reduction targeting household-level storage practices. This pattern echoed findings from other endemic provinces where water insecurity drives container-based storage, inadvertently sustaining *Aedes* spp. proliferation (Espino et al., 2013). These persistent domestic and peridomestic breeding sources could be addressed by integrating environmental management and behavioral change communication into existing Department of Health strategies, such as the enhanced 4S approach (“Search and Destroy,” “Seek Early Consultation,” “Self-Protection,” and “Say Yes to Fogging During Outbreaks”).

The absence of dengue virus detection in adult mosquito pools likely reflects the timing of specimen collection relative to infection cycles, low virus prevalence at the sampling window or RNA degradation due to silica-based transport (Bangs et al., 2007). Although the latter is a known limitation of dry preservation, this issue is better framed as an operational constraint rather than methodological error. Future field investigations should consider using RNA-preserving solutions (such as RNAlater) to maintain viral integrity during transport.

While the larval indices did not differ significantly between case and non-case households, the lower PPI and AHI values among the case-history households suggested that immediate post-outbreak interventions (such as fogging and container clean-up) had localized effects in suppressing vector density. Nevertheless, the persistence of vectors after interventions may signal partial coverage or emerging insecticide resistance—an issue corroborated by recent findings of pyrethroid resistance in *Ae. aegypti* populations from urban sites in the Philippines (Malijan et al., 2024). Critical actions to maintain control efficacy include sustained monitoring of resistance status and rotation of insecticide classes, as outlined in various guidelines (World Health Organization, 2009, 2016; Insecticide Resistance Action Committee, 2007, 2019).

The primary limitation of the current study was the cross-sectional, two-day survey design, which restricted temporal interpretation. In addition, volunteer-assisted sampling may have introduced variability despite pre-field standardization. More comprehensive risk modeling could be achieved by expanding future work to include longitudinal monitoring, insecticide resistance bioassays and meteorological correlates, such as rainfall and temperature lag effects, as was demonstrated recently in long-term dengue surveillance studies in the Philippines (Cruz et al., 2024).

Overall, the current findings validated the use of integrated entomological indices to capture both larval and adult dynamics during outbreaks by highlighting the urgent need for sustained integrated vector management (IVM) combining environmental sanitation, chemical and biological control and community participation—approaches endorsed under the World Health Organization Global Vector Control Response 2017–2030 (World Health Organization, 2017).

Conclusion

This field investigation confirmed that the 2023 dengue outbreak in Araceli, Palawan, the Philippines was accompanied by exceptionally high *Aedes* vector densities, with all measured indices exceeding national and World Health Organization risk thresholds. Both traditional and expanded metrics indicated intense transmission potential driven by prolific breeding in outdoor water storage containers. Although no dengue virus was detected in adult pools, the sustained presence of *Aedes aegypti* and *Aedes albopictus* signifies a persistent risk for re-emergence.

To strengthen outbreak prevention and response, it is imperative: 1) to incorporate pupal and adult surveillance into routine entomological monitoring; 2) to intensify source reduction targeting household water storage practices; 3) to implement continuous insecticide resistance monitoring and strategic insecticide rotation; and 4) to reinforce the Department of Health’s 4S Strategy through community-based environmental management and behavioral change communication.

Integrating these actions within an IVM framework should enhance early detection, rapid response and sustainable control of dengue vectors in the Philippines.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

- Aktar, W., Sengupta, D., Chowdhury, A. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* 2: 1–12. doi.org/10.2478/v10102-009-0001-7
- Bangs, M.J., Pudiantari, R., Gionar, Y.R. 2007. Persistence of dengue virus RNA in dried *Aedes aegypti* (Diptera: Culicidae) exposed to natural tropical conditions. *J. Med. Entomol.* 44: 163–167. doi.org/10.1603/0022-1589-44.1.1632585(2007)44[163:PODVRI]2.0.CO;2
- Carvalho, G.C., da Silva, M.A.N., Zeppelini, C.G., de Lima, J.B.P. 2014. Morphological differentiation of *Aedes aegypti* and *Aedes albopictus* pupae using paddle and terminal abdominal segment characters. *Parasit. Vectors.* 7: 232. doi.org/10.1186/1756-3305-7-232
- Chang, M.S., Hii, J.L. 2011. Vector surveillance and control in the prevention and control of vector-borne diseases: An integrated approach. *Trop. Biomed.* 28: 1–17.
- Cruz, E.I., Salazar, F.V., Aguila, A.M.A., Villaruel-Jagmis, M.V., Ramos, J. and Paul, R.E. 2024. Current and lagged associations of meteorological variables and *Aedes* mosquito indices with dengue incidence in the Philippines. *PLoS Negl. Trop. Dis.* 18: e0011603. doi.org/10.1371/journal.pntd.0011603
- Department of Health. 2018a. Manual of procedures for the national *Aedes*-borne viral disease prevention and control program (NAVDPCP). Volume 1: Program overview. A step-by-step guide to the interventions related to the prevention and control of the three *Aedes* mosquito-borne viral diseases in the Philippines: Dengue, Zika and Chikungunya. Department of Health, the Philippines.
- Department of Health. 2018b. Manual of procedures for the national *Aedes*-borne viral disease prevention and control program (NAVDPCP). Volume 3: Integrated vector management. A step-by-step guide to the interventions related to the prevention and control of the three *Aedes* mosquito-borne viral diseases in the Philippines: Dengue, Zika and Chikungunya. Department of Health, the Philippines.
- Department of Health. 2018c. Manual of procedures for the national *Aedes*-borne viral disease prevention and control program (NAVDPCP). Volume 2: Vector, virus and case surveillance. A step-by-step guide to the interventions related to the prevention and control of the three *Aedes* mosquito-borne viral diseases in the Philippines: Dengue, Zika and Chikungunya. Department of Health, the Philippines.
- Espino, F., Marco, J., Salazar, F., Salazar, N., Mendoza, Y., Velazco, J. 2013. Community-based dengue vector control: Experiences in behavior change in Metropolitan Manila, Philippines. *Pathog. Glob. Health.* 107: 373–379. doi.org/10.1179/2047773213Y.0000000115
- Focks, D.A. 2003. A review of entomological sampling methods and indicators for dengue vectors. World Health Organization. Geneva, Switzerland.
- Garcia, G.A., Hoffmann, A.A., Maciel-de-Freitas, R., Villela, D.A.M., Codeço, C.T. 2018. *Aedes aegypti* insecticide resistance underlies the success (and failure) of Wolbachia population replacement. *Sci. Rep.* 8: 6889. doi.org/10.1038/s41598-018-25347-2
- Insecticide Resistance Action Committee. 2007. Prevention and management of insecticide resistance in vectors and pests. <https://www.irac-online.org>, 20 November 2025.
- Insecticide Resistance Action Committee. 2019. IRAC guidelines: Prevention and management of resistance in vectors. <http://www.irac-online.org>, 20 November 2025.
- Johnson, B.W., Russell, B.J., Lanciotti, R.S. 2005. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J. Clin. Microbiol.* 43: 4977–4983. doi.org/10.1128/JCM.43.10.4977-4983.2005
- Malijan, M.A.R., Salazar, F.V., de la Cruz, J.T., Cruz, M.A.B., Baje, F.M.A., Madarieta, M.R.G., Amalin, D.M. 2024. Insecticide resistance in *Aedes aegypti* in selected urban sites of the Philippines: Implications for vector control. *Parasit. Vectors.* 17: 15. doi.org/10.1186/s13071-024-05973-7
- Ong, E.P., Obeles, A.J.T., Ong, B.A.G. and Tantengco, O.A.G. 2022. Perspectives and lessons from the Philippines' decades-long battle with dengue. *Lancet Reg. Health West. Pac.* 24: 100505. doi.org/10.1016/j.lanwpc.2022.100505
- PrimerDesign. 2023. Multiplex for dengue, chikungunya and Zika virus v2 genesig® kit handbook. <https://www.primerdesign.co.uk/media/downloads/bbd34216-018c-11ef-bcea-fa163e309ced.pdf>, 20 November 2025.
- Provincial Health Office–Palawan. 2023. Dengue outbreak report, Araceli municipality. Palawan Provincial Health Office, the Philippines.
- QGIS Development Team. 2025. QGIS Geographic Information System. QGIS.org Association, Solothurn, Switzerland. <http://qgis.org>
- Qiagen 2023. QIAamp® Viral RNA Mini handbook. <https://www.qiagen.com/us/resources/download.aspx?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en>, 20 November 2025.
- Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E. 2005a. Illustrated keys to the mosquitoes of Thailand. II. Genera *Culex* and *Lutizia*. *Southeast Asian J. Trop. Med. Public Health.* 36: 1–97.
- Rattanarithikul, R., Harrison, B.A., Harbach, R.E., Panthusiri, P., Coleman, R.E. 2005b. Illustrated keys to the mosquitoes of Thailand. I. Background; genus *Aedes*, subgenus *Stegomyia*. *Southeast Asian J. Trop. Med. Public Health.* 36: 1–80.

- Rattanakrithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Jones, J.W., Coleman, R.E. 2006. Illustrated keys to the mosquitoes of Thailand. IV. Genera *Anopheles*, *Chagasia* and *Bironella*. Southeast Asian J. Trop. Med. Public Health. 37: 1–128.
- Rattanakrithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P. and Coleman, R.E. 2007. Illustrated keys to the mosquitoes of Thailand V. Genera *Orthopodomyia*, *Kimia*, *Malaya*, *Topomyia*, *Tripteroides* and *Toxorhynchites*. Southeast Asian J. Trop. Med. Public Health 38: 1–65.
- Rattanakrithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E. and Richardson, J.H. 2010. Illustrated keys to the mosquitoes of Thailand VI. Tribe Aedini. Southeast Asian J. Trop. Med. Public Health 41: 1–225.
- Rueda, L.M. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa. 589: 1–60.
- R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Sparks, T.C., Nauen, R. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121: 122–128. doi.org/10.1016/j.pestbp.2014.11.014
- Sternberg, E.D., Thomas, M.B. 2018. Insights from agriculture for the management of insecticide resistance in disease vectors. Evol. Appl. 11: 404–414. doi.org/10.1111/eva.12501
- World Health Organization. 1995. Vector surveillance and control at ports, airports and ground crossings. World Health Organization Technical Report Series No. 818. <https://iris.who.int/server/api/core/bitstreams/ce651999-5a9d-4a23-adb5-c0d707e630d4/content>
- World Health Organization. 2003. Guidelines for dengue surveillance and mosquito control (2nd ed.). World Health Organization Regional Office for the Western Pacific. <https://iris.who.int/items/9ddd6003-035d-4d04-a472-328d1971fcfe>, 20 November 2025.
- World Health Organization. 2009. Handbook for integrated vector management. World Health Organization Press. <https://apps.who.int/iris/handle/10665/44768>, 20 November 2025.
- World Health Organization. 2011. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. World Health Organization Regional Office for South-East Asia. <https://iris.who.int/items/f3f4cbe8-1718-40d2-a750-c4d9480e707b>, 20 November 2025.
- World Health Organization. 2016. Monitoring and managing insecticide resistance in *Aedes* mosquito populations: Interim guidance for entomologists. <https://apps.who.int/iris/handle/10665/204588>, 20 November 2025.
- World Health Organization. 2017. Global vector control response 2017–2030. World Health Organization. <https://www.who.int/publications/i/item/9789241512978>, 20 November 2025.
- World Health Organization. 2023. Western Pacific Region Dengue Situation Update 2023. World Health Organization Western Pacific Surveillance Bulletin. <https://iris.who.int/server/api/core/bitstreams/92a7492a-2f27-42e7-be3f-de7ad05c8819/content>, 20 November 2025.