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Original Article

Integrated analysis of dengue transmission risk and insecticide resistance in *Aedes aegypti* from Vichada, Colombia

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Abstract

Dengue is the primary arbovirus transmitted by *Aedes aegypti* mosquitoes. Effective management of dengue demands a multidisciplinary approach. The aim of this study was to conduct an integrated analysis of dengue transmission, focusing on its vector mosquitoes, to establish a baseline for dengue control and prevention in an endemic region of Colombia. The study was conducted from 2015 to 2018 across four municipalities in the Vichada department near the Venezuelan border. Five complementary approaches were employed: (1) determining the natural infection rate and circulation of various dengue virus serotypes in mosquitoes; (2) evaluating the insecticide susceptibility status and examining mosquito genotypes for three knockdown (*kdr*) mutations linked to insecticide resistance; (3) performing a phylogenetic analysis to identify the lineage of *Ae. aegypti*; (4) creating risk maps for dengue transmission based on predictive models in two municipalities in the department; and (5) empowering the community. Molecular analysis using RT-PCR indicated dengue virus infections in all municipalities of the Vichada department. Eleven positive pools for serotypes DENV-1, DENV-2, DENV-3, and DENV-4 were detected. The highest minimum infection rate (MIR) was found in Cumaribo, followed by La Primavera, which showed the greatest diversity of dengue virus serotypes. Mosquitoes from all three populations showed susceptibility to malathion and lambda-cyhalothrin pyrethroid, except for mosquitoes from Puerto Carreño, which presented moderate resistance to lambda-cyhalothrin (resistance ratio of 8). Importantly, all mosquitoes had F1534C mutation, while the V1016I and V419L mutations were found at lower frequencies. Risk classification maps for Puerto Carreño and La Primavera showed neighborhoods with high risk, indicating potential hotspots for intervention and vector control. This study established a necessary baseline for the ongoing monitoring and improvement of the early warning system for all municipalities in the Vichada department. The integrative approach employed in this study highlights the importance of incorporating these methodologies into dengue epidemiological surveillance in endemic regions.

Keywords: Dengue, *Aedes aegypti*, insecticide resistance, *kdr* mutations, vector surveillance

Introduction

Arboviruses such as dengue, Zika, yellow fever, and chikungunya impact millions of people, especially in the tropical and subtropical regions of Asia, Africa, and the Americas, where warm temperatures and humidity facilitate the proliferation of the primary vector, *Aedes* (*Stegomyia*)

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aegypti [1,2]. Climate change, migration, and merchandise distribution encourage insect colonization in non-endemic areas like Europe and North America, thereby increasing the risk in these regions. In the Andean region of South America, Colombia is one of the countries most affected by these arboviruses [3], primarily due to the widespread presence of *Ae. aegypti*. Social factors that generate unplanned urbanization, high population movement, changes in domestic water usage, and a lack of public health policies to implement effective control measures [4,5] also contribute to the spread of these vector-borne diseases.

In Colombia, the number of reported cases over the last five years (2019–2023) has increased by 300% compared to the previous year [6,7]. This trend continued into 2024, with cases surging dramatically (https://portalsivigila.ins.gov.co, consulted on August 30, 2024). The eastern region of the country, which borders Venezuela, includes the department of Vichada, where dengue transmission is both endemic and epidemic, exhibiting cyclical behavior with outbreaks occurring in 1990, 1993–1994, 1996, 2001, and 2003; along with significant epidemics in 2006, 2010, 2019, and 2023 [8]. Data from the national surveillance system reported a total of 1,644 dengue cases between 2007 and 2020 (https://portalsivigila.ins.gov.co, consulted on August 30, 2024). During epidemiological week 35 of 2024, nearly 625 cases were reported, mainly in the Cumaribo municipality, accounting for approximately 80% of those cases (https://portalsivigila.ins.gov.co) [9]. Organophosphate and pyrethroid insecticides are the most common strategies for reducing mosquito populations [10]. However, a general issue that hinders successful mosquito control is the need to understand the insecticide sensitivity status of *Ae. aegypti* in regions with high arbovirus transmission. Furthermore, resistance to these insecticides has been documented in various areas of the country, complicating mosquito control efforts in other regions [10].

Furthermore, the current high migration flow in this department requires the implementation of a virological surveillance system for *Ae. aegypti*. This system would help identify and monitor new cases and serotypes [11]. Regular mosquito virological surveillance supports informed decision-making for interventions and forms the basis for creating an early warning system that can forecast epidemics.

Overall, effective dengue management requires a multidisciplinary approach involving various strategies. Using molecular techniques to detect the dengue virus in field-collected mosquitoes provides valuable entomological information, as it identifies microfoci of transmission with greater precision and enables vector control measures that mitigate the disease's impact. Similarly, studying insecticide susceptibility profiles and identifying resistance mechanisms are critical for implementing effective control strategies. The aim of this study was to perform an integrated analysis of dengue transmission, focusing on its vector *Ae. aegypti* in the Vichada department to establish a baseline for dengue control and prevention. For this purpose, five approaches were used: (1) determination of the natural infection rate and circulation of different dengue virus serotypes; (2) evaluation of the insecticide susceptibility status of *Ae. aegypti* and mosquito genotypes to search for three knockdown (*kdr*) mutations (V419L, V1016I, and F1534C) associated with insecticide resistance; (3) phylogenetic study to identify the lineage of *Ae. aegypti*; (4) construction of risk maps for the transmission of dengue from predictive models in two municipalities of the department; and (5) providing community training to strengthen local capacity through molecular biology methods for diagnosing natural *Ae. aegypti* infections and developing bioassays with insecticides.

Methods

Study area

This study was conducted from 2015 to 2018 in the Vichada department, located in eastern Colombia, on the border with Venezuela. The department is divided into four municipalities: Puerto Carreño, La Primavera, Santa Rosalía, and Cumaribo. In Puerto Carreño, the capital, the collection took place in December 2015 in urban areas covering nine neighbourhoods (6° 11' 24.99" N; 67° 28' 46.03" W) and in the rural zone Casuarito (5° 40' 51.41" N; 67° 38' 38.36" W). In 2016, between March and April, La Primavera and Santa Rosalía were visited, and eight neighborhoods (5° 29' 22.10" N; 70° 24' 53.24" W) and two settings (5° 8' 29.98" N; 70° 51' 30.76" W) were sampled. Finally, in March 2017, the collection was carried out in five neighborhoods of Cumaribo

(4° 26' 47.50" N; 69° 47' 41.15" W) (**Figure 1**). For all municipalities, every house in each neighborhood was studied.

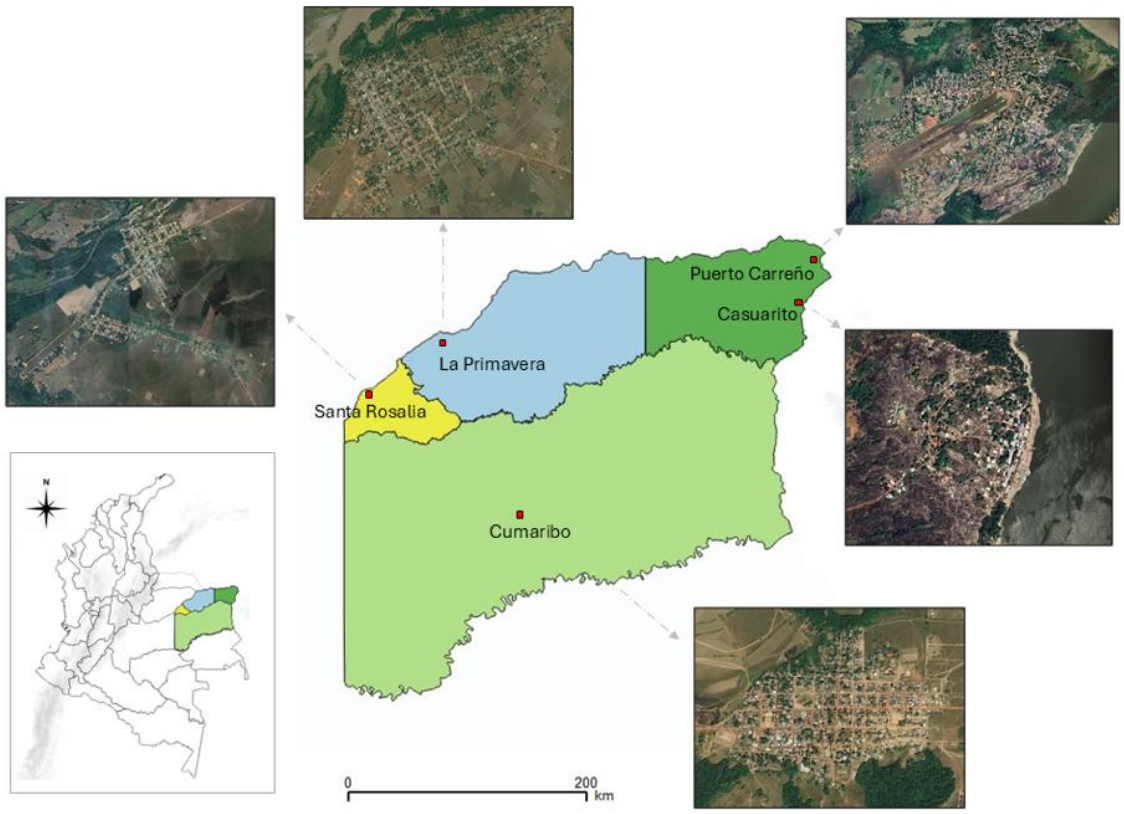

Figure 1. Map of Colombia showing the Vichada department and the municipalities of Puerto Carreño, La Primavera, Santa Rosalía, and Cumaribo.

Mosquito collection, rearing, identification, and colony establishment

Adults, pupae, larvae, and ovitraps were collected by health staff from each municipality involved in vector-borne disease programs. During home visits, a thorough search for adults was conducted for 15 minutes using entomological nets and a mouth aspirator in bedrooms, bathrooms, living rooms, and other areas where homeowners reported the presence of mosquitoes, such as pools, low tanks, and storage tanks for drinking water. The collected samples were kept alive in a plastic container and transported to the Biología y Control de Enfermedades Infeciosas (BCEI) laboratory at the University of Antioquia. The immature stages were reared to adulthood (F0 generation) and maintained under controlled conditions of temperature (28°C ± 1°C), relative humidity (80% ± 5%), and photoperiod (12 h light : 12 h dark). The collected material was taxonomically identified following Rueda's morphological key [12]. Three *Ae. aegypti* colonies were established using the F0 generation mosquitoes: Puerto Carreño, La Primavera, and Cumaribo. The F1 mosquitoes were used to carry out all the experiments.

Extraction and amplification of viral RNA

RNA extraction was conducted in pools ranging from 1 to 20 mosquitoes collected from the field (F0 adults) via the commercial RNeasy Mini Kit (Qiagen, Hilden, Germany). Each pool was mechanically macerated following the manufacturer's instructions. One-step RT-PCR was performed with the Luna® Universal One-Step RT–qPCR Kit (Biolabs Inc., Ipswich, MS, USA) to detect Zika, chikungunya, and the four dengue virus serotypes. Each 10 µL reaction contained 1 µL of RNA, 1× enzyme mixture, 1× reaction mixture, and 0.4 µM of each primer. The RT-PCR thermal cycling protocol involved reverse transcription at 55°C for 10 min, initial denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 10 sec, 56°C for 30 sec, and 72°C for 20 sec. RNA from the supernatant of cell cultures infected with each dengue serotype, Zika, and chikungunya viruses served as positive controls. Amplification products were analyzed on 2.5% agarose gels in 0.5× TBE

buffer, stained with 1× GelRed, and visualized under ultraviolet (UV) light using a photographic documentation system (Bio-Rad®, California, USA). All primers and product sizes are listed the **Table S1** (see **Underlying data**).

Bioassays with insecticides

Larvae

Larval bioassays were conducted using the standardized methodology of the World Health Organization (WHO) (1981) [13]. Lambda-cyhalothrin, with 97.8% purity (SIGMA-ALDRICH, Burlington, MA, USA), was diluted in ethanol to 1 mL to prepare a stock solution at 100 ppm. From this concentration, various solutions were prepared that caused mortality rates ranging from 2% to 99% of the larvae. Six insecticide concentrations were tested to determine the LC_{50} and LC_{90}, and confidence intervals (CIs) were calculated for each mosquito population. A total of 20 third- and fourth-instar larvae of *Ae. aegypti* from the F1 generation of each strain (Puerto Carreño, La Primavera, and Cumaribo) were used for the bioassay. As a control, the insecticide-susceptible Rockefeller laboratory *Ae. aegypti* strain was used and compared across three independent tests. The control treatments consisted of 1% ethanol, and larval mortality was recorded after 24 hours of exposure.

The percentage of larvae mortality at each concentration after 24 hours was analyzed to calculate the LC_{50} using log-probit regression with the SPSS software (IBM Corp., Armonk, USA) version 27.0 [14]. The resistance ratio (RR) was calculated by dividing the LC_{50} of the population under study by that obtained for the susceptible Rockefeller reference strain. Field populations are considered moderately resistant to a given insecticide when their RRs are greater than those of the Rockefeller strain [15]. The statistical significance ($p<0.05$) of differences in LC_{50} between strains was assessed using the Lethal Dose Ratios test [16]. This method involves the overlap of the 95% CI for each strain compared with the 95% CI of the Rockefeller strain. Non-overlapping CIs indicate a significant difference with $p<0.05$. Resistance levels were categorized as susceptible (<5 times), moderately resistant (5 to 10 times), and resistant (>10 times) [17].

Adults

Bioassays were conducted following the methodologies described by the Centers for Disease Control and Prevention (CDC) in Atlanta, United States. Adult females of the F1 generation were obtained from each of the *Ae. aegypti* strains and subjected to bioassays using the impregnated bottle method. The bioassay employed malathion with a purity of 98.7% (Fluka Analytical, cat. 36143, St. Louis, USA), which was diluted in absolute ethanol (Merck 99.9% purity, Darmstadt, Germany) to a concentration of 50 ppm. For the bioassay development, 20 females aged 3–5 days from the filial generation F1 and without blood feeding were introduced into 250 mL Schott® bottles previously impregnated with 1 mL of the insecticide or absolute ethanol in the case of controls. The number of live and dead mosquitoes was recorded after 30 minutes of exposure [18]. Three biological repetitions were carried out for this test, each consisting of three technique replicates plus an ethanol control. The Rockefeller strain was used as a control to determine the diagnostic dosage and time following previously described procedures [19]. All the bioassays were performed under controlled laboratory conditions at an average temperature of 26°C and a relative humidity of 70%.

The analysis of adult bioassay results was conducted following the mortality criteria outlined in the CDC methodology (Instructions for the Evaluation of Insecticide Resistance in Vectors through the CDC Bottle Biological Assay). If mortality is less than 80%, the population is resistant; if mortality is 98% or higher, the population is susceptible; and if mortality falls between 80% and 97%, the population is considered to have the potential for resistance and should be monitored closely.

DNA extraction and kdr mutation genotyping

At least 30 field-collected adult mosquitoes and laboratory-emerged larvae were selected for genomic DNA extraction. Mosquitoes were individualized in 1.5 mL tubes, and total DNA was extracted via the ZR Tissue & Insect DNA Miniprep Kit (ZYMO RESEARCH, California, USA). The DNA was subsequently resuspended in 50 µL of water. Two allele-specific PCR (AS–PCRs) were performed for each mosquito under the same conditions to identify the V419L, V1016I, and F1534C

mutations in the sodium channel gene, as was previously reported [19]. The amplification products were analyzed on 1.5% agarose gel electrophoresis (Sigma Aldrich Co., St. Louis, USA).

Amplification and sequencing of the cytochrome oxidase I (COI) gene

The COI mitochondrial marker was amplified in 64 *Ae. aegypti* mosquitoes from the Vichada department via the primers reported in the **Underlying data** and previous research [20]. Polymerase chain reaction (PCR) amplification was performed with 2 µL of template DNA, 5 µL of 10× reaction buffer (Thermo Fisher, Waltham, MA, USA), 0.2 mM dNTPs, 20 pmol of each primer, and 1 U of Taq polymerase (Thermo Fisher, Waltham, MA, USA). The reaction was conducted at 94°C for 30 s, 48°C for 30 s, and 72°C for 1 min, followed by 10 min at 72°C. The PCR products were detected via agarose gel electrophoresis. All positive PCR products were purified and sequenced via the Sanger method at the Macrogen Sequencing Service in Seoul, South Korea.

Polymorphism and phylogeographic analysis

The sequences were aligned via CLUSTAL W, as implemented in BioEdit 7.1.9 (North Carolina State University, North Carolina, USA) [30]. Genetic variability was assessed by calculating the number of haplotypes (h), haplotype diversity (Hd), and nucleotide diversity (π). Tajima's D test [33] was performed to evaluate whether all the mutations were selectively neutral. Genetic differentiation among populations was assessed via the Fst index. All polymorphism analyses were conducted in DnaSP v.6.12.03 (University of Barcelona, Barcelona, Spain). The Vichada haplotypes were analyzed phylogeographically by comparing them to 298 mosquito additional sequences from the Antioquia, Meta, and La Guajira departments, which were previously reported in Colombia (GenBank accession codes KM203140—KM203248). The analysis was performed via a median-joining haplotype network constructed with Network v.4.6.1.1 software (Fluxus Technology Ltd., Clare, UK).

Epidemiological data and risk classifications

Information on *Aedic indices* registered by health secretaries from 2001 to 2015 was used to determine the risk classifications for dengue in each locality, along with dengue epidemiological data obtained from SIVIGILA (Sistema Nacional de Vigilancia en Salud Pública, Colombia) for the years 2008 to 2015. Dengue cases and *Aedic indices* were georeferenced based on patients' addresses, images, and CAD files containing the political division of each locality. The software ArcGIS (Esri, New York, USA) was used to analyze this information. The local indicators of spatial association (LISA) were used to determine whether a region has high or low risk, as reported previously, considering the population reported for each neighborhood, the number of cases in each zone, and the *Aedic indices* known [21]. LISA can identify five classes of spatial grouping: (1) HH, which corresponds to a zone with high risk surrounded by zones with high risk; (2) LH, which is a zone with low risk surrounded by zones with high risk; (3) LL, zones with low risk surrounded by zones with low risk; (4) HL, zones with high risk surrounded by zones with low risk; and (5) zones with nonsignificant correlations among each vicinity region.

Community training program

This study provided a training program on dengue and tropical diseases to staff from the Puerto Carreño health secretary, which is the capital of the Vichada department. The training was held at the University of Antioquia in Medellín and at the health secretary's office in Puerto Carreño. This included generating risk maps and geographic information systems (GIS) for the Departmental Health Secretariat, along with training on assessing susceptibility to pyrethroids and organophosphates using CDC and WHO guidelines for entomology department staff. Additionally, training on serological diagnosis of other tropical diseases was provided. Participants were selected based on their experience with tropical diseases and received one month of laboratory training at the University of Antioquia.

Results

Dengue serotypes in *Aedes aegypti* from the department of Vichada

A total of 107 female *Ae. aegypti* mosquitoes were collected in Puerto Carreño and Casuarito, along with 30 females in La Primavera and Santa Rosalía, and 76 females in Cumaribo. A total of 64 *Ae.*

aegypti pools were analyzed for natural infection. Molecular analysis by RT-PCR revealed dengue virus infection in all municipalities of the Vichada department. Surprisingly, eleven positive pools for serotypes DENV-1, DENV-2, DENV-3, and DENV-4 were identified (**Table 1**). The highest minimum infection rate (MIR) was found in Cumaribo, followed by La Primavera, which showed the greatest diversity of dengue serotypes.

Table 1. Dengue serotypes in *Aedes aegypti* females pooled from municipalities of the Vichada department, Colombia

Municipality	*Aede aegypti*			Minimum infection rate (MIR)	Identified dengue serotype
	Female pools collected	Females pool processed	Positive females pool		
Puerto Carreño	101	18	1	55.55	DENV-4
Casuarito	6	6	1	166.67	DENV-1
La Primavera	15	15	4	266.67	DENV-1, DENV-3, DENV-4
Santa Rosalia	15	15	1	66.67	DENV-4
Cumaribo	76	10	4	400	DENV-1, DENV-2, DENV-3
Total	213	64	11	171.87	DENV-1, DENV-2, DENV-3, DENV-4

Susceptibility of mosquito populations to insecticides

Mosquitoes from all three populations (Puerto Carreño, La Primavera, and Cumaribo) presented a susceptibility profile to Malathion, with a mortality rate of 100% of the individuals at the time of diagnosis, which was 30 minutes (**Figure 2A**). Similarly, mosquitoes from La Primavera and Cumaribo were susceptible to lambda-cyhalothrin pyrethroid, with RR of 4 and 6, while the Puerto Carreño *Ae. aegypti* population showed a RR of 8, indicating one moderate resistance to this insecticide (**Figure 2B** and **Table S2** (see **Underlying data**)). Overlapping, 95% of CIs between La Primavera, Cumaribo, and Puerto Carreño populations were observed, indicating a non-significant difference. However, the mosquito populations differed significantly from the Rockefeller susceptible strain (**Table S2**).

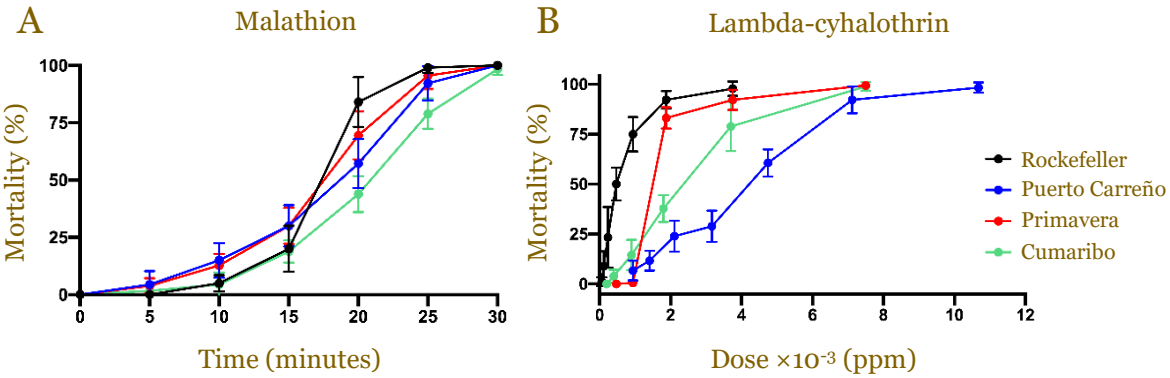

Figure 2. Susceptibility of mosquitoes from Puerto Carreño, La Primavera, and Cumaribo municipalities to malathion (A) and lambda-cyhalothrin (B) insecticides.

Frequencies of *kdr* mutations across mosquito populations in Vichada

Fifty-eight *Ae. aegypti* mosquitoes from Puerto Carreño were processed for resistance-associated mutations in the sodium channel gene (*kdr* mutations). At least 39 mosquitoes were evaluated for each mutation (**Table 2**). Notably, all the mosquitoes presented the F1534C mutation. Conversely, the V1016I and V419L mutations were observed at low frequencies.

Table 2. Allele frequency of V1016I, V419L, and F1534C mutations in mosquitoes collected in the Vichada department

Mutation	n	Allelic frequency		Genotype		
		Wild type	Mutated	Wild type homozygous	Heterozygous	Mutated homozygous
1016	53	0.98	0.02	0.96 (50)	0.04 (3)	0

Mutation	n	Allelic frequency		Genotype		
		Wild type	Mutated	Wild type homozygous	Heterozygous	Mutated homozygous
419	58	0.97	0.03	0.95 (56)	0.05 (2)	0
1534	39	0	1	0	0	1

Polymorphism and phylogeographic relationships of *Ae. aegypti* from Vichada

A 660 bp fragment was analyzed in 64 individuals from Vichada, revealing 31 haplotypes with 51 variable sites (7.72%), of which 15 (29.41%) were parsimony informative and 36 (70.58%) were singleton sites (**Table 3**). The overall haplotype diversity (Hd) was 0.704±0.066, and the nucleotide diversity (π) was 0.004±0.001. Both haplotype and nucleotide diversities were higher in La Primavera and Santa Rosalía than in Cumaribo (**Table 3**). Tajima's D tests revealed negative and significant values (p<0.05) in La Primavera and Santa Rosalía, indicating that these populations are undergoing selective processes (**Table 3**).

Table 3. Genetic diversity and Tajima's D neutrality tests in *Ae. aegypti* from Vichada based on the *COI* gene (the sequences are available on GenBank, accession numbers PV034731.1 to PV034794.1)

Municipality	n	h	Hd±SD	π±SD	D
La Primavera	25	15	0.817±0.081	0.005±0.001	-2.579***
Santa Rosalia	25	14	0.813±0.080	0.005±0.001	-1.842***
Cumaribo	14	2	0.143±0.119	0.001±0.001	-1.155
Total	64	31	0.704±0.066	0.004±0.001	-2.584***

D: Tajima's D test; h: haplotypes observed; Hd: haplotype diversity; SD: standard deviation; π: nucleotide diversity
*Statistically significant at p=0.05

Low Fst (<0.05) indicates gene flow among populations, whereas moderate values (0.05–0.15) suggest limited genetic differentiation. Gene flow analyses revealed moderate genetic differentiation among Cumaribo and Santa Rosalía (Fst=0.096) and low genetic differentiation among Cumaribo and La Primavera (Fst=0.011) and La Primavera with Santa Rosalía (Fst=0.032).

The haplotypic network revealed many low-frequency haplotypes belonging to two main groups previously reported in Colombia. Group 1 included sequences from Vichada and those from Antioquia, Meta, and La Guajira, whereas Group 2 was restricted to Antioquia and La Guajira. The most frequent haplotype, H1, was found across all departments, with a frequency of 0.620 (**Figure 3**).

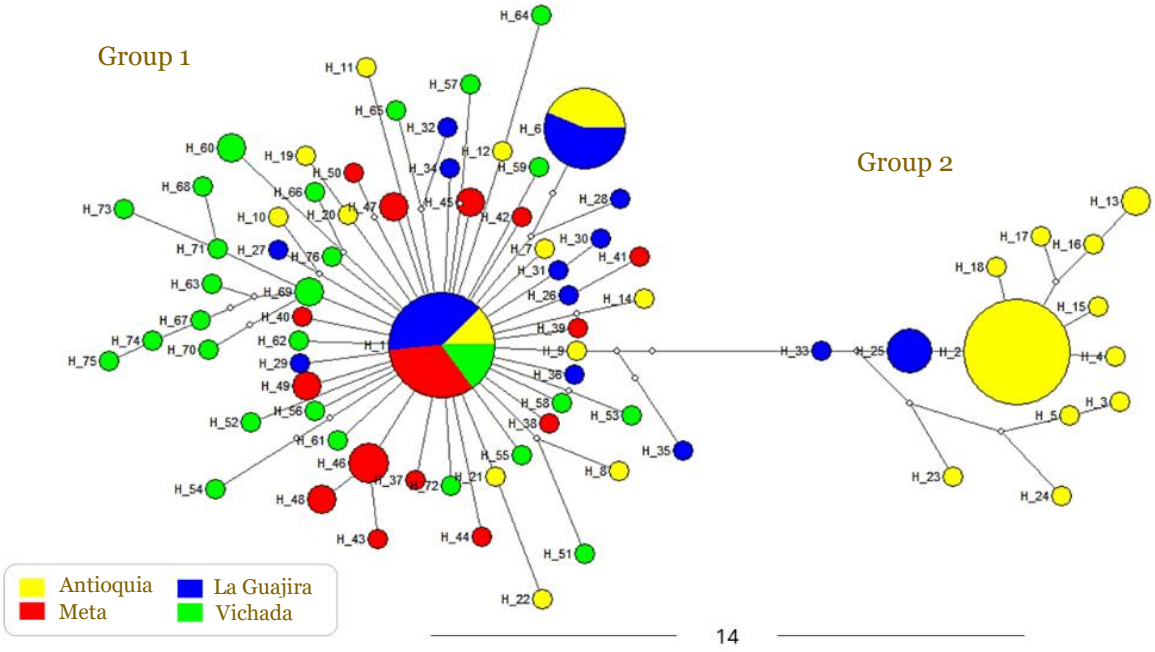

Figure 3. Phylogeographic relationships among 76 haplotypes of Colombian *Ae. aegypti* based on the median-joining haplotype network of the *COI* gene. The size of the nodes corresponds to the haplotype frequency; white nodes indicate median vectors (hypothetical haplotypes). The black bar denotes the number of mutational steps (14 steps) between the nodes of group 1 (left) and group 2 (right).

Risk classification

Risk classification maps were generated for Puerto Carreño and La Primavera based on entomological and epidemiological data from 2008–2015 (**Figures 4A** and **4B**). Cumaribo and Santa Rosalía data were unavailable because this information was absent in these cities. The classification risk via LISA for each neighborhood in Puerto Carreño is presented in **Table S6** (see **Underlying data**). Notably, some neighborhoods showed high risk (HH). These HH neighborhoods represent potential hotspots for intervention and should be prioritized for vector control.

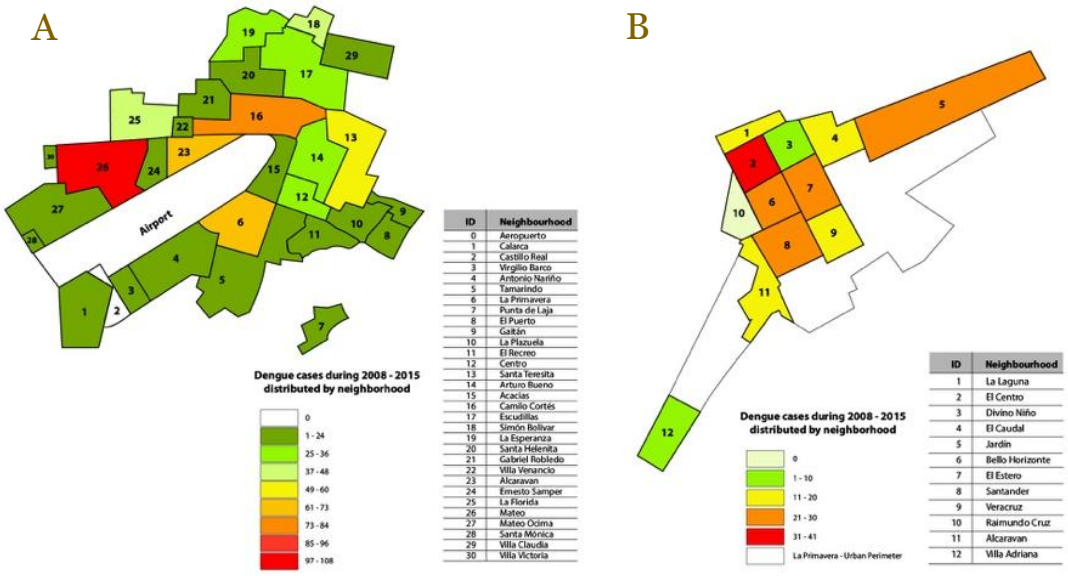

Figure 4. Risk map for the dengue virus in Puerto Carreño (A) and La Primavera (B) municipalities based on data from 2008 to 2015.

Community training program

The program emphasized serological diagnosis of vector-borne diseases (VBDs), including dengue, Chagas disease, and leishmaniasis; developing home enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) tests; and using molecular techniques for diagnosis. Additionally, the team received training on generating risk maps and GIS. After each activity, feedback was provided, detailing the results obtained, and a report on the staff's field application was developed.

Discussion

Dengue is the fastest-growing mosquito-borne disease, with 3.9 billion people in 128 countries at risk of viral infection and 96 million cases estimated annually [22,23]. Two insect vectors transmit the dengue virus, *Ae. aegypti* and *Ae. albopictus*, which also transmits Zika, chikungunya, and other viruses [6,22,24]. Colombia, infested in almost 80% of its territory by *Ae. aegypti* and *Ae. albopictus*, is one of the most affected tropical regions [6,7]. The country has several regions with serious dengue problems where the disease is considered endemic-epidemic, such as the Department of Vichada. However, owing to the violence of illegal groups, difficulties in mobility, precarious living conditions, high migratory flow, and other factors, few studies have comprehensively addressed the problem of dengue fever and its vectors throughout departments.

This study addressed the dengue issue by examining five key aspects related to the epidemiology of the disease in the sampling area: the natural infection of mosquitoes with dengue serotypes; insecticide resistance and potential resistance mechanisms found in the sampled mosquitoes; mosquito lineages and their distribution within the study area; the distribution of cases and risk maps; and finally, community empowerment.

First, a baseline of mosquito natural infection was established in all the municipalities that comprise the department to have enough elements for constructing a surveillance program to help prevent outbreaks throughout the department. Among the most relevant results were the natural

mosquito infection rate of 40% in Cumaribo and the circulation of the DENV-1, DENV-2, and DENV-3 serotypes. This raises the possibility of secondary infections that are typically associated with severe dengue cases. Surprisingly, the natural infection rate found for this municipality is much higher than those reported elsewhere in Colombia during the same epidemiological periods, such as Tolima (33%), Valle del Cauca (12.7%), Bello (16.8%), Riohacha (9.62%), and Medellin (32.42%) [25–27]. Furthermore, the findings for Cumaribo reinforce its classification as a high-risk area for transmission within the department of Vichada, with an incidence rate of 329 per 100,000 inhabitants (www.portalsivigila.ins.gov.co, consulted on August 30, 2024).

Moreover, our results revealed an infection rate of 26.6% in *Ae. aegypti* females and the cocirculation of DENV-1, DENV-3, and DENV-4 serotypes in La Primavera. The National Institute of Health from Colombia (INS) has classified this municipality as having a medium risk of dengue transmission, with an incidence rate of 195.9 per 100,000 inhabitants (www.portalsivigila.ins.gov.co, consulted on August 30, 2024). The findings also indicated that the natural infection rate of *Ae. aegypti* in this municipality was higher than that reported in municipalities of the Orinoquia region, such as Villavicencio, which recorded an incidence rate of 466 per 100,000 inhabitants, but had a natural infection rate of only 11.8 [28]. These results highlight the need for ongoing virological surveillance, especially considering alerts may be triggered if DENV-2 is detected. La Primavera's geographical position facilitates the movement of people from Casanare, Meta, and Arauca; therefore, establishing surveillance in this municipality is a priority for developing an early warning system.

Beyond infection patterns, our findings also reveal insights into vector control through insecticide profiles. The susceptibility tests for larvicides and adulticides (pyrethroids and organophosphates) indicated that the *Ae. aegypti* populations from Vichada are susceptible to malathion insecticide. However, the mosquito populations showed moderate resistance to the lambda-cyhalothrin insecticide. This finding aligns with the low frequency of at least two mutations, 1016I and 419L. Notably, the F1534C mutation was found in all the mosquitoes. The fixation of F1534C suggests widespread historical exposure to pyrethroids, although its presence alone may not confer high resistance levels. To date, three *kdr* mutations have been identified in the sodium channel gene coding region of *Ae. aegypti* in Colombia, which are associated with resistance to pyrethroids. Mutations V1016I and F1534C have been reported in populations exhibiting pyrethroid resistance traits along the Caribbean coast, in the coffee region, Meta and Antioquia. More recently, the V419L mutation was linked to lambda-cyhalothrin resistance in Riohacha and Villavicencio populations [19]. In light of these findings, we emphasize several critical points and recommendations for this Colombian region. First, while the mutated alleles are rare (I1016=0.02 and L419=0.03), these mutations could become fixed due to insecticide usage. Consequently, it is recommended that the Secretary of Health avoid extensive and uncontrolled insecticide applications and educate the community that trained professionals should only conduct such actions. Second, regular monitoring of populations is crucial to determine whether mutation frequencies and resistance levels are increasing, which could trigger alerts regarding control methods. Third, it is essential to implement spraying with a rotation of insecticides between pyrethroids and organophosphates to prevent insecticide resistance while incorporating additional control strategies.

The organophosphate malathion susceptibility test showed that the three *Ae. aegypti* strains were susceptible to this insecticide. Similar findings have been reported in Antioquia, Putumayo, Chocó, Nariño, Cauca, Atlántico, Casanare, and Caldas departments [29-32]. However, losses in susceptibility to this insecticide, linked to its extensive use, have been reported in Colombia and other countries on the continent [31,33,34]. Therefore, monitoring insecticide susceptibility in this region should be conducted continuously.

Another interesting aspect to study is the knowledge of the population structure of *Ae. aegypti*, which is essential for improving prevention strategies in endemic areas. Molecular studies of the NADH, ND4, and *COI* genes suggested that *Ae. aegypti* comprises two ancestral clades, a basal clade associated with mosquitoes from West Africa and a second clade derived from the first, consisting of mosquitoes from East Africa [28]. These differences have important epidemiological implications because changes in specific characteristics, such as vector competence for dengue virus and insecticide resistance between the two clades, have been reported. In Colombia, the

presence of two clades of *Ae. aegypti* mitochondrial or ancestral lineages have been reported. The first is widely distributed and has a more significant presence in cities with the highest incidence of dengue. The second virus was recently introduced to the country and has been reported in areas with a low incidence of this virus [35]. The results presented here make this the first study to evaluate the molecular and phylogeographic diversity of *Ae. aegypti* in the Department of Vichada. *COI* mitochondrial gene sequence analysis revealed the exclusive presence of haplogroup 1 derived from West Africa in the Department of Vichada and low genetic structure among municipalities, indicating continuous genetic flow between them. Given its prevalence in other high-incidence areas, this haplogroup's dominance in Vichada warrants further investigation into a potential link with vector competence.

Colonization processes often involve a small group of individuals founding a new population, leading to reduced genetic diversity compared to the source population. This reduction occurs due to sampling effects, where only a subset of genotypes from the original population establishes the new population [36]. The exclusive presence of haplogroup 1 across all municipalities and individuals genetically similar to those from Villavicencio. These findings suggest a likely origin from central Colombia, though comparative data from neighbouring countries like Venezuela is needed to confirm this hypothesis.

On the other hand, the risk maps generated for the city of Puerto Carreño and the municipality of La Primavera can become useful decision-making tools for planning intervention actions for these two populations. Modified indices can detect spatial and differential risks for both epidemic and endemic periods, allowing early detection of dengue outbreaks in both populations. The maps generated could predict risk at the spatiotemporal level in endemic years. They could be incorporated into surveillance activities in endemic locations, such as the municipalities for which they were constructed.

Although knowledge of dengue diagnosis, mosquito identification and typing, characterization of *kdr* mutations, and insecticide bioassays increased after training, establishing and implementing a comprehensive dengue program for the entire department is essential to ensure the sustainability of surveillance, prevention, and control efforts. While promising, future evaluations should determine whether trained personnel continue to apply these skills and if local surveillance indicators improve over time.

This study is limited by the relatively short sampling period, the lack of concurrent human case data, and incomplete risk mapping data in some municipalities. Future longitudinal studies integrating entomological and clinical surveillance are needed.

Conclusion

This study generated the baseline necessary to continue monitoring and strengthening the early warning system for all municipalities in the Vichada department. To our knowledge, this is the first integrated entomological and molecular study conducted in this area, offering valuable insights into local dengue transmission dynamics. Key findings included a notably high natural infection rate in Cumaribo, the susceptibility of mosquito populations to malathion, and the presence of the F1534C resistance mutation in all tested mosquitoes. The results can inform targeted insecticide use, prioritize high-risk areas for intervention, and support training programs for local entomological surveillance. In this sense, the integrative approach used in this study supports the need to incorporate these methodologies into dengue epidemiological surveillance in endemic regions to develop a more robust and efficient surveillance and control program for dengue and other arboviruses transmitted by *Ae. aegypti*.

Ethics approval

All animals used in this study were handled strictly in good animal practice under the Colombian Code of Practice for the Care and Use of Animals for Scientific Purposes, established by Law 84 of 1989. Ethical approval (Act No 2223, 2018) was obtained from the Animal Ethics Committee of Antioquia University, Medellin, Colombia.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author upon reasonable request. The supplementary data can be accessed through the following link: http://dx.doi.org/10.6084/m9.figshare.30644522.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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