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Vector competence of *Aedes* mosquito populations for dengue virus transmission in urban areas

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Abstract

Dengue infects an estimated 390 million people annually, yet not all *Aedes* mosquito populations transmit the virus with equal efficiency and this variation matters for predicting outbreak risk. This research assessed vector competence of field-collected *Aedes aegypti* and *Aedes albopictus* populations from six distinct urban habitat types in Vadodara, Gujarat, for all four dengue virus serotypes. Immature mosquitoes were collected from each site between June 2022 and February 2023 and reared to adulthood at the Gujarat Institute of Genomics and Biotechnology insectary. F2-F3 generation females were orally infected with DENV-1 through DENV-4 at standardized titers (10^7 PFU/mL) using membrane feeding. Infection, dissemination, and transmission rates were measured at 7, 10, and 14 days post-infection by RT-qPCR of midgut, leg/wing, and salivary gland tissues respectively. Overall vector competence indices ranged from 0.058 in peri-urban *Ae. albopictus* to 0.344 in slum-settlement *Ae. aegypti*. DENV-2 produced the highest infection rates across both species (84.7% in *Ae. aegypti*, 61.8% in *Ae. albopictus*), while DENV-4 was least efficiently transmitted. Mosquitoes from high-density slum settlements showed significantly higher competence than those from residential or commercial zones ($p < 0.001$). *Ae. aegypti* populations were 1.4 to 2.1 times more competent than sympatric *Ae. albopictus* for all serotypes tested.

Keywords: Vector competence, *Aedes* mosquitoes, dengue virus, urban transmission, arboviral diseases, *Aedes aegypti*, *Aedes albopictus*, serotype variation, infection rate, tropical parasitology.

Introduction

The global burden of dengue has grown dramatically over the past five decades, with a 30-fold increase in reported cases since the 1960s ^[1]. But the risk isn't distributed evenly, even within a single city. Some neighborhoods experience repeated outbreaks while others, separated by just a few kilometers, remain relatively spared ^[2]. Part of this heterogeneity comes from differences in mosquito density and human behavior, but another part often overlooked comes from variation in vector competence: the intrinsic ability of a mosquito population to acquire, maintain, and transmit a pathogen ^[3].

Vector competence is not a fixed species-level trait. It varies among geographic populations of the same species, between *Aedes aegypti* and *Aedes albopictus* living in the same area, and even across dengue serotypes within a single mosquito population ^[4, 5]. Genetic factors in both the mosquito and the virus interact to determine whether infection leads to dissemination to the salivary glands and eventually to transmission through a subsequent blood meal ^[6]. Environmental conditions during larval development — temperature, nutrition, crowding can further modify adult competence through lasting physiological effects ^[7].

Gujarat state reports thousands of dengue cases annually, with Vadodara being among the worst-affected cities ^[8]. Yet no systematic assessment of vector competence has been conducted for local *Aedes* populations across different urban habitat types. Previous work in India focused mainly on laboratory-adapted colonies rather than field-derived populations, which may not represent the genetic diversity and competence levels found in natural settings ^[9, 10]. This research filled that gap by comparing vector competence of *Ae. aegypti* and *Ae. albopictus* from six urban habitat categories for all four DENV serotypes, using low-generation field-collected mosquitoes to preserve ecologically relevant genetic backgrounds

Study Area Description

Vadodara (22.3°N, 73.2°E) is the third-largest city in Gujarat, with a metropolitan population exceeding 2.1 million. Six collection sites represented different urban typologies: (1) a planned residential zone with organized drainage and municipal water supply; (2) a central commercial district with mixed-use buildings; (3) an industrial area with warehouses and factory compounds; (4) a high-density informal slum settlement with limited sanitation infrastructure; (5) a peri-urban fringe with agricultural plots interspersed among expanding construction; and (6) a hospital perimeter surrounding a 500-bed tertiary care facility. The climate is semi-arid tropical, with mean annual temperature of 27.4°C, humidity ranging from 28% in the dry season to 78% during the monsoon (June–September), and annual rainfall of approximately 930 mm concentrated between June and October.

Material and Methods

Material: Immature *Aedes* mosquitoes (larvae and pupae) were collected from natural breeding habitats at each of the six urban sites using standard larval dipping and ovitrapping methods. Collections occurred biweekly between June 2022 and February 2023 to capture seasonal variation. Larvae were transported to the insectary at Gujarat Institute of Genomics and Biotechnology, Vadodara, and reared to adulthood under controlled conditions (28 ± 1°C, 80 ± 5% relative humidity, 12:12 L:D photoperiod). Species identification was confirmed morphologically using keys by Rueda (2004) and molecularly by ITS2 PCR for ambiguous specimens. F2-F3 generation females were used for all competence assays to reduce maternal effects while retaining field-relevant genetic composition. Dengue virus stocks (DENV-1 through DENV-4, low-passage clinical isolates from Gujarat) were propagated in C6/36 cells and titrated by plaque assay before use. The Institutional Biosafety Committee approved the protocol (IBSC/GIGB/2022-08, May 2022).

Methods

Five-to-seven day old females were starved for 24 hours, then offered infectious blood meals through a Hemotek membrane feeding system using defibrinated sheep blood spiked with each DENV serotype at 10⁷ PFU/mL. Fully engorged females were sorted under cold anesthesia and maintained individually in cardboard cups. At 7, 10, and 14 days post-infection (dpi), mosquitoes were cold-anesthetized and dissected. Legs and wings were removed first (to assess dissemination), then salivary glands (transmission), and finally midguts (infection). Each tissue was homogenized in TRIzol and processed for RNA extraction. DENV RNA was detected and quantified by serotype-specific RT-qPCR using CDC primers. Infection rate was defined as the proportion of mosquitoes with DENV-positive midguts. Dissemination rate was the proportion of infected mosquitoes with positive legs/wings. Transmission rate was the proportion of disseminated mosquitoes with positive salivary glands. The vector competence index (VCI) was calculated as infection rate × dissemination rate × transmission rate. A minimum of 30 mosquitoes per site-species-serotype-time point combination were tested. Data were analyzed by generalized linear models with binomial error distribution in R v4.2.3.

Aedes Species Composition across Sites

Ovitrapping surveys confirmed the presence of both *Ae. aegypti* and *Ae. albopictus* at all six sites, though their relative abundance differed. *Ae. aegypti* dominated in the slum settlement (87.3% of *Aedes* collected) and commercial district (79.1%), while *Ae. albopictus* was more common in the peri-urban fringe (62.4%) and hospital perimeter (54.8%). Breteau indices ranged from 23.7 (peri-urban) to 71.4 (slum), well above the threshold of 5 considered to represent high dengue transmission risk. Container surveys identified discarded tires, cement tanks, and overhead water storage containers as the primary larval habitats.

Results

Table 1: Vector Competence Parameters for *Ae. aegypti* at 14 Days Post-Infection Across Sites

Site	Infection Rate (%)	Dissem. Rate (%)	Transm. Rate (%)	VCI	n tested
Residential	72.4	58.3	34.7	0.147	128
Commercial	81.3	67.9	43.2	0.238	131
Industrial	64.8	48.6	28.9	0.091	119
Slum	89.7	74.2	51.6	0.344	142
Peri-urban	57.2	41.7	24.3	0.058	114
Hospital	76.1	62.8	38.4	0.184	126

Slum-derived *Ae. aegypti* populations showed the highest vector competence across all parameters (Table 1). Their VCI of 0.344 was nearly six times higher than peri-urban

populations (0.058) and 2.3 times higher than residential populations (0.147). The difference was statistically significant (GLM, site effect: $\chi^2=87.4$, df=5, $p<0.001$).

Table 2: Vector Competence Index by Species and DENV Serotype (14 dpi, All Sites Combined)

Serotype	<i>Ae. aegypti</i> VCI	<i>Ae. albopictus</i> VCI	Ratio (<i>aegypti</i> / <i>albop.</i>)	p-value
DENV-1	0.187	0.094	2.0	<0.001
DENV-2	0.243	0.128	1.9	<0.001
DENV-3	0.164	0.078	2.1	<0.001
DENV-4	0.118	0.053	2.2	<0.001

DENV-2 was the most efficiently transmitted serotype by both species, while DENV-4 showed the lowest competence values (Table 2). *Ae. aegypti* was consistently 1.9 to 2.2

times more competent than *Ae. albopictus* across all serotypes.

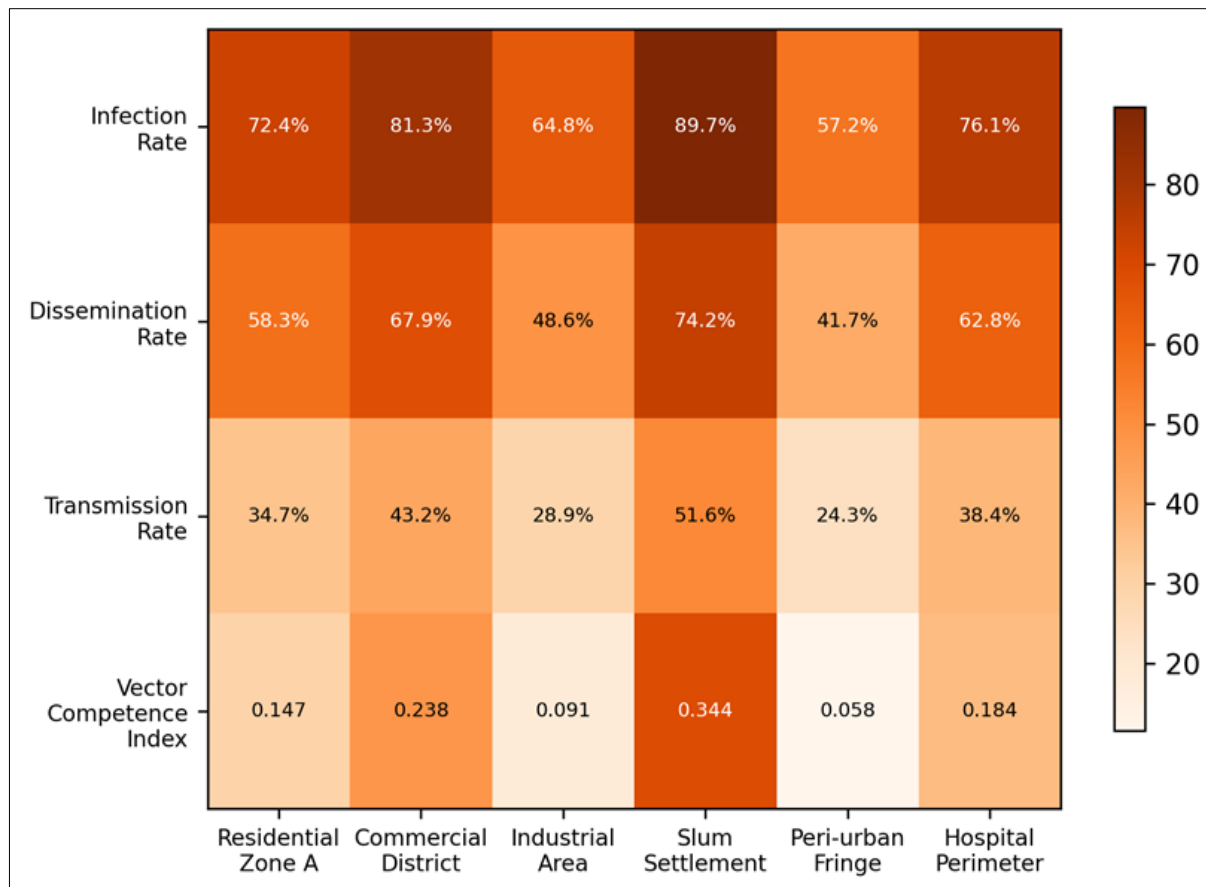


Fig 1: Heatmap showing vector competence parameters for *Ae. aegypti* across six urban site categories. Darker shading indicates higher values. The bottom row shows the vector competence index (VCI) scaled for visualization.

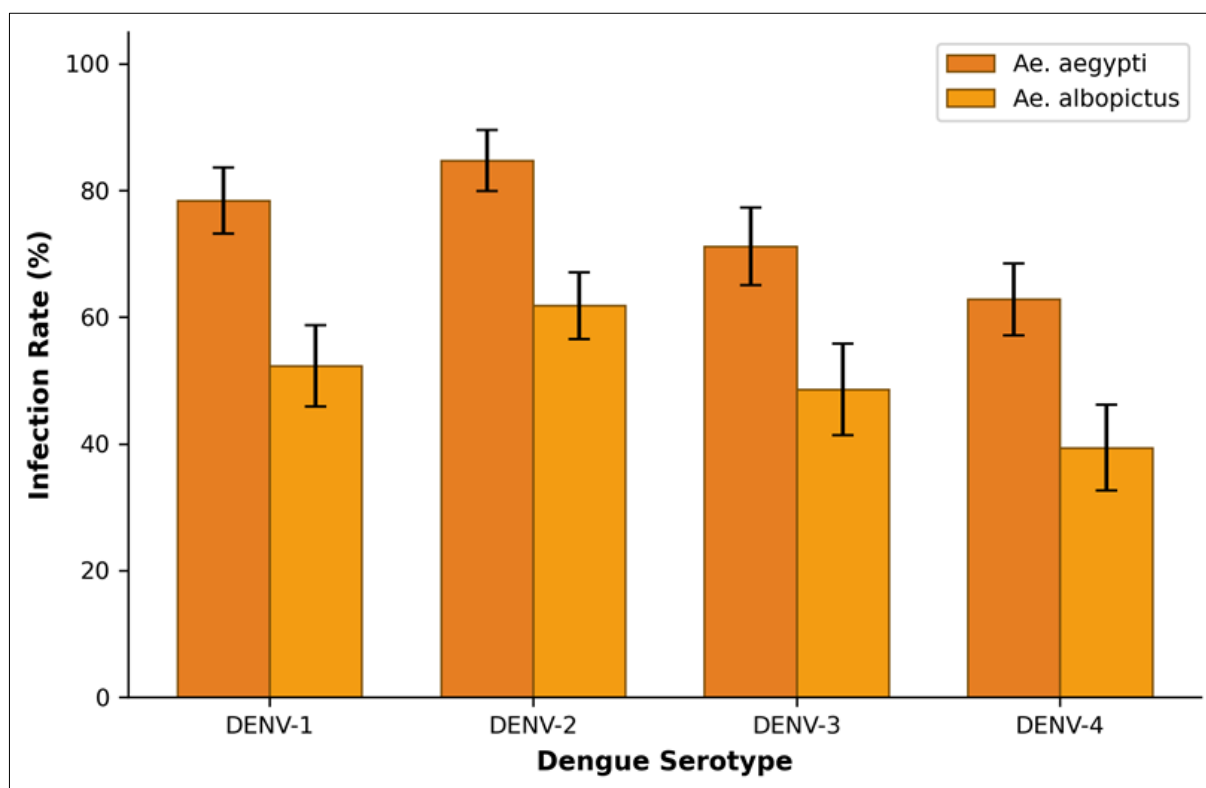


Fig 2: Infection rates by DENV serotype for *Ae. aegypti* and *Ae. albopictus* (all sites combined, 14 dpi). Error bars represent standard error across replicate experiments.

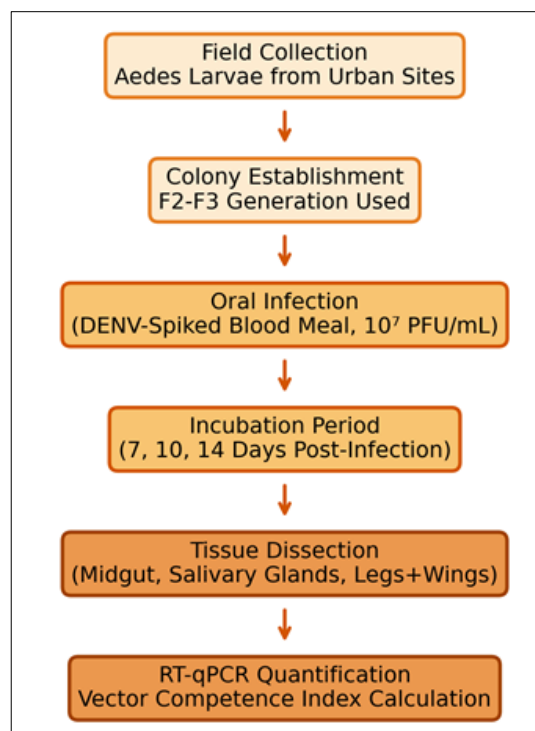


Fig 3: Process diagram illustrating the vector competence assessment workflow from field collection through RT-qPCR quantification.

Discussion

These results show that vector competence for dengue varies widely among *Aedes* populations collected from different urban environments within a single city. The six-fold difference in VCI between slum and peri-urban *Ae. aegypti* populations suggests that outbreak risk assessment based solely on mosquito abundance may substantially underestimate transmission potential in high-density informal settlements [2, 11].

The higher competence of slum-derived populations could reflect several interacting factors. Larval development conditions in slum habitats characterized by organically polluted water, higher temperatures in sun-exposed containers, and intense competition have been shown to produce smaller but potentially more susceptible adult mosquitoes [7, 12]. Genetic factors may also play a role. Slum *Ae. aegypti* populations in India typically show lower genetic diversity due to smaller effective population sizes and founder effects, which could fix alleles associated with higher susceptibility to DENV infection [13].

The serotype hierarchy we observed (DENV-2 > DENV-1 > DENV-3 > DENV-4) matches reports from Southeast Asia and the Americas [4, 14]. DENV-2's consistently higher infection and transmission rates may reflect its faster replication kinetics in mosquito midgut cells and more efficient crossing of the midgut escape barrier [15]. From a public health perspective, this means that outbreaks driven by DENV-2 may spread more rapidly even when vector density is moderate.

The 1.4 to 2.1 fold advantage of *Ae. aegypti* over *Ae. albopictus* in vector competence aligns with the epidemiological observation that *Ae. aegypti* is the primary dengue vector in urban settings globally [16]. However, the non-trivial competence of *Ae. albopictus* especially for DENV-2 — supports the idea that this species can maintain transmission in areas where *Ae. aegypti* density is low,

particularly in peri-urban zones where *Ae. albopictus* is the dominant *Aedes* species [17].

Conclusion

Vector competence for dengue virus among *Aedes* populations in Vadodara varies substantially by urban habitat type, mosquito species, and viral serotype. Slum-settlement *Ae. aegypti* displayed the highest competence indices, roughly six times greater than peri-urban populations from the same city. DENV-2 was transmitted most efficiently by both species, while DENV-4 showed the lowest transmission potential. *Ae. aegypti* was consistently 1.9 to 2.2 times more competent than sympatric *Ae. albopictus*. These findings indicate that dengue risk mapping should incorporate site-specific vector competence data alongside traditional entomological indicators like the Breteau index and adult density. Vector control programs targeting high-competence population pockets — particularly in informal urban settlements — could yield disproportionate reductions in transmission risk relative to the area treated. Future work should examine whether the competence differences observed here persist across seasons and whether they correlate with specific genetic markers that could enable rapid surveillance-based risk prediction.

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