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Research Article

A Preliminary Survey on Geospatial Distribution of *Aedes albopictus* and *Aedes aegypti* Mosquitoes Found in Different Urban and Rural Locations of Southern West Bengal, India

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ABSTRACT

Aedes mosquitoes are globally known to transmit various arboviral diseases including Dengue fever. Historically considered an urban disease, Dengue fever has been reported in rural areas of different countries also. Two important species, *Aedes aegypti*, and *Aedes albopictus*, are rapidly spreading and invading new locations in India and risking human health more frequently each year. Since vector control has been proven to be the most effective measure to prevent such disease, knowing the vectors more, would help design successful methods to fight against the disease. In this survey, a cross-sectional inspection was conducted to look for the existence of *Aedes* spp. at 420 locations including urban and rural areas of 15 districts in Southern West Bengal, India, Morphological identification of collected specimens, comparative analyses of vector distribution and their breeding habitats, between districts, rural and urban locations as well as between different physiographic regions in this area were performed. The study revealed that sixty-four percent of the urban locations inspected and 22% of the rural locations inspected were positive for *Aedes* spp. Despite the region's physiographic, climatic, and anthropogenic diversity, the districts shared a similar pattern of species distribution - *A. albopictus* was found in both rural and urban locations of all the districts while *A. aegypti* was observed in only urban locations of thirteen districts. In some locations, both species were found to be co-existing in the same habitat containers. Mean larval density and mean container index were both higher in urban habitats than in rural areas though few rural locations showed significantly high density of larval population. The current findings of this survey thus provide a comprehensive picture of Aedes distribution in the said area and may in turn act as a foundation for more effective surveillance to characterize these vectors and to identify potential dengue outbreak hotspots in Southern West Bengal.

Keywords: Aedes aegypti, Aedes albopictus, Distribution, Rural, South West Bengal, Urban

Introduction

Adult *Aedes* female mosquitoes spread several viral diseases like Dengue fever, Chikungunya, and Zika. [1,2]. *Aedes aegypti* and *Aedes albopictus* are known to be potential vectors of dengue epidemics [3]. These two species have been prevalent in India since the middle of the nineteenth century and pose a risk to human health [4,5,6]. In the past ten years, dengue cases have increased rapidly throughout the nation. According to the National Vector Borne Disease Control Program (NVBDCP) report of 2021, a total of 193245 dengue cases occurred in India of which 346 patients expired.

A. aegypti, the primary vector for dengue, whose ancestral forms resided in sub-Saharan African forests, has invaded the Gangetic plains of

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North India. This species exhibits indoor resting behavior and prefers urban areas [7,8]. In contrast, *A. albopictus*, formerly known as the forest mosquito of Southeast Asia, now lives all across the country in a wide range of habitats, including urban areas, farmland, or deep forest. *A. albopictus* usually rests outdoors and is an opportunistic feeder [9, 10, 11]. Several findings confirm the occurrence of dengue fever in rural areas [12, 13], though it was more commonly designated as an urban disease. Factors like globalization, rapid urbanization, human population movement, and climatic shifts have created new habitats, expanded the geographic range of the species, and paved the way towards domestication of these mosquito species as well [14,15]. Since vaccines or specific medications for arboviral diseases are not yet effective [16], integrated vector management in India plays the most important role in preventive and control measures. This management includes identification of the species responsible, reducing their breeding habitats, using larvicides, and using anti-adult measures to kill the different stages of mosquitoes [17, 18, 19]. In addition, by analyses of variables like larval density and the Berteau index, it may also be possible to predict future dengue outbreaks at any location [20].

In West Bengal, an eastern state of India, a significant number of Dengue fever cases are observed every year, mostly during the post-monsoon season. Reports from various new locations of the state, more noticeably from the Southern districts, are increasing [6, 16, 21]. This tropical region, which mainly comprises Gangetic plains and delta, has hot summers, moderate rainfall, and dry winter weather. This climate provides ideal breeding environments for *Aedes* mosquitoes and, as a consequence, creates an incremental potential threat. Southern Bengal also includes the highlands of the western plateau with moderately different weather. A transitional region known as Rarh Bhumi lies in between the two mentioned regions. The plateau shows comparatively less rainfall, occasional drought, and low temperatures during winters as compared to the plains [22, 23].

Previous reports, mainly from hot and humid urban locations of some districts of West Bengal, confirmed the existence of the two vector species, *A. aegypti*, and *A. albopictus*, in this region [24, 25, 26, 27, 28]. In our present survey, we included both rural and urban locations of each district to look for the distribution of these two species in a much bigger and diverse region that included 420 locations covering 15 districts of Southern West Bengal, India.

Material and Methods

Study area selection

This study took place in fifteen districts (Figure 1) of Southern West Bengal, India (21˚38´36´´N – 24˚37´05´´N, 85˚48´35´´E – 88˚59´12´´E) an area of about 65,051 sq. km. The region has been divided broadly into three physiographic divisions - 1. Western plateau and highland, 2. Transitional Rarh Bhumi/ Rarh Bengal and 3. Gangetic plain, delta, and coastal region in the east and south. These divisions show significant diversity in several geographical parameters like elevation, temperature, relative humidity, average annual rainfall, and anthropogenic factors like human population density, occupation, and awareness [22, 23, 29, 30]. Four hundred and twenty locations (Figure 1C), including a total of 2200 premises, were inspected. For each district, a comparable number of urban and rural locations were chosen. The selection of locations was random, but for each district, we tried to maintain a similar physical distance (minimum 8 km) between two locations, and the dengue reports of previous years [6, 30] were also considered. Official criteria from the Census 2011 Govt. of India data were used to distinguish between urban and rural locations [29]. An average of five premises per location were randomly selected, and an average of three probable breeding habitats per premise were studied.

During the survey of private properties, verbal permission from the landlord or head of the house was obtained prior to entering their property. The collection sites included semi-permanent indoor and outdoor water storages filled with stagnant rain or domestic water.

Specimen collection and morphological identification of mosquitoes

The survey was conducted in two phases, one from June 2019 to March 2020 and another from May 2021 to August 2022. Immature stages of mosquitoes (larvae or pupae) were collected and brought to the laboratory in plastic containers. Collection and total count calculation of larvae and pupae were done following the standard method by using dippers [31, 32]. The definite proportion of larvae from each location was examined morphologically for species-level identify-

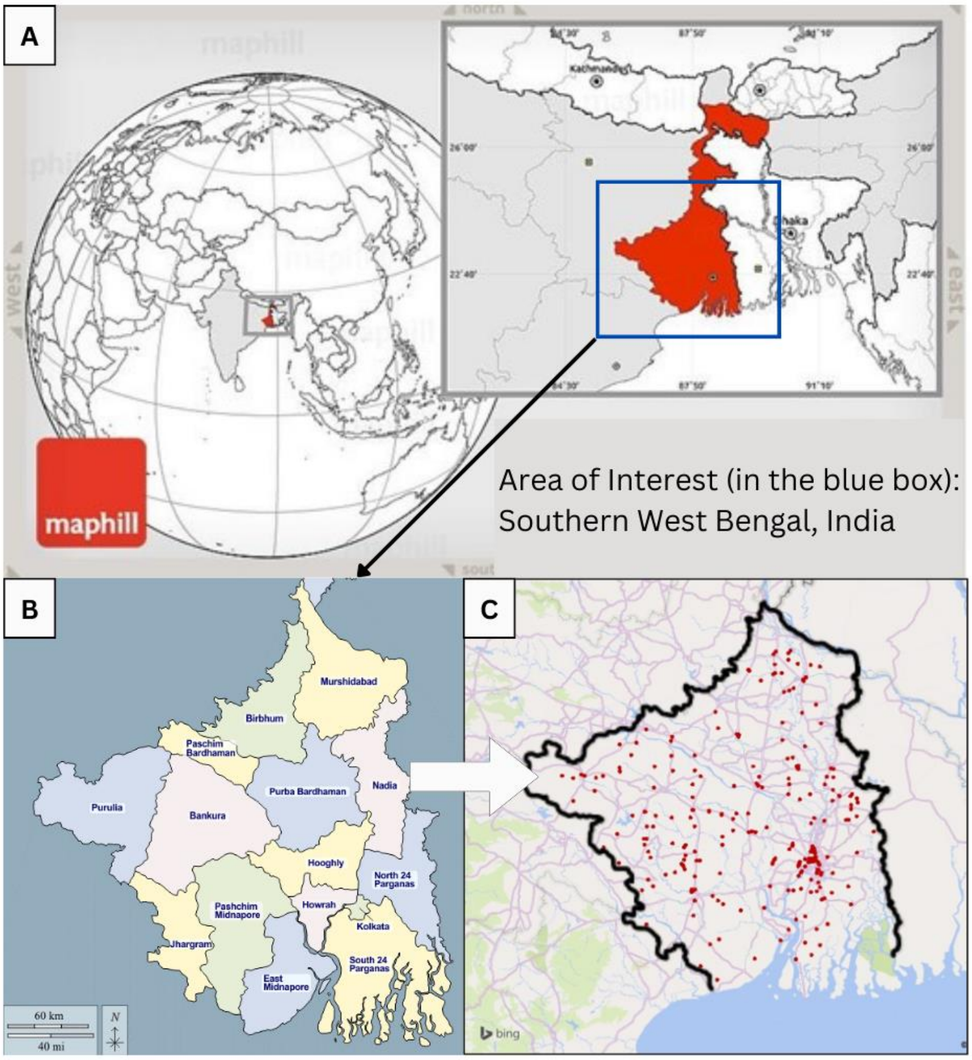

Figure 1. Area of survey. A. West Bengal, India (in red; Source: maphill). The southern part of the state is in blue rectangle. B. The fifteen districts of south West Bengal. C. Map showing distribution of locations (red dot coded; inclusive of mosquito positive and negative locations) inspected for *Aedes* mosquito in the study area (created by MS-Excel 3D map).

cation following standard taxonomic methods [33,34] using appropriate taxonomic keys. Some larvae were allowed to develop into (F_0) adults in insect cages and 2-3 days old adults were killed with ether and used for morphological identification. Biosafety measures were always adopted during the whole process of collection and maintenance of mosquitoes.

Habitat analysis

During a visual inspection at the locations, data on different habitat characteristics like – area type (rural/urban), container/habitat type (tire, basin, pots or tree trunk), location (outdoor/indoor), and water source (rain/domestic) were documented.

Statistical analysis

Different characteristics of the larval population (like total count and density) were calculated. Comparative analyses were done for various locations between districts, between the three different physiographic regions, as well as between urban and rural locations within the same districts. The recorded data were tabulated using MS excel-2019. Since the data fulfills the normality assumptions, parametric statistical tests were chosen for analysis. Statistical analyses were done using R software (Version- 2022.07.2). Guidelines of NVBDCP [17] for larval surveys, like calculating larval density and container index, were followed with a few modifications.

$$Larva\ density = \frac{Larva\ present}{100\ mL\ of\ habitat\ water}$$

$$Container\ index = \frac{number\ of\ positive\ containers}{number\ of\ containers\ inspected} \text{x}100$$

Results and Discussion

Species identification, abundance, and its distribution

The tropical climate of Southern West Bengal, India, with its variety of vegetation and moderate amount of rainfall, provides favourable breeding sites for several mosquito species. The present study has focused on the identification of Aedes species and their breeding habitats in fifteen districts of Southern Bengal so that targeted preventive measures can be planned to combat Dengue and other arboviral diseases in this area. A total of 60,000 immature stages of *Aedes* (57060 in urban locations and 2940 in rural locations) were studied. Of the 420 locations surveyed, 195 locations were positive for the presence of larvae/pupae. We observed the presence of *Aedes aegypti* and *Aedes albopictus,* the two species known to be the most prevalent dengue vectors in India.

Morphological identification and pictorial documentation of larvae and emerged adults revealed that of the total 60,000 larvae, 31,950 were *A. albopictus* and 28,050 were *A. aegypti.* Sixty-four percent of the total urban locations studied were positive for the presence of either or both mosquito species whereas about 22% of the total rural locations studied were positive for *A. albopictus. A. albopictus* is more widely distributed at several urban and some rural locations in the fifteen districts surveyed whereas *A. aegypti* was found in all the districts except Birbhum and Bankura (Figure 2) but seemed to be restricted to urban locations.

During 1969 - 1973, an extensive survey took place in search of these two vectors in and around cities of many physiographic regions of India, including the Bengal Gangetic basin and the eastern Chhota Nagpur plateau. Though confined to the urban cities, the study inferred that during that period of time, *A. aegypti* (the most effective vector) was quite stable in the large towns of the "Bengal basin", but *A. albopictus* was found in a negligible number in peripheral areas [5]. In contrast, later observations revealed the existence of *A. albopictus* in the cities and their peripheries in high density, co-existing and competing *with A. aegypti.* Tandon and Raychoudhury (1998) stated that dengue used to be transmitted by *A. aegypti* in cities and *A. albopictus* in suburban (urban peripheries) and rural areas. But an invasion of suburban and urban areas by *A. aegypti* and *A. albopictus* respectively, seem to have taken place. This is probably due to change in epidemiology of dengue and the rapid increase of favourable breeding habitat that has occurred as a result of rapid urbanisation, easy transportation and global warming [36]. Our finding supports this later trend, in different urban

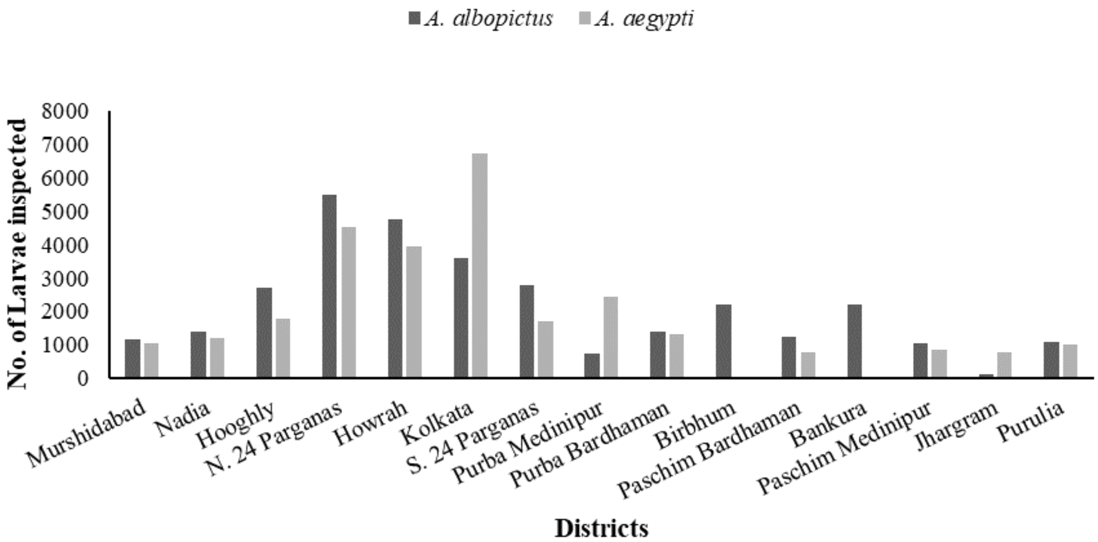

Figure 2. Abundance of *Aedes albopictus* and *Aedes aegypti* mosquitoes (total number of larvae inspected) in 15 districts of Southern West Bengal.

Table 1. Larval abundance, mean larval density and container index (CI) for *A. albopictus* and *A. aegypti* in rural and urban area surveyed

Area type	Species	Total larval count	Mean larval density (Mean±SE)	Container index (Mean±SE)
Rural	*Aedes albopictus*	2940	9.7±1.12	2.67±0.45
	Aedes aegypti	0	0	
Urban	*Aedes albopictus*	29010	36.61±3.59	35.13±1.72
	Aedes aegypti	28050	39.46±5.97	

Here, for larval density calculation only mosquito-positive locations were considered but for container index calculation both positive and negative containers were included.

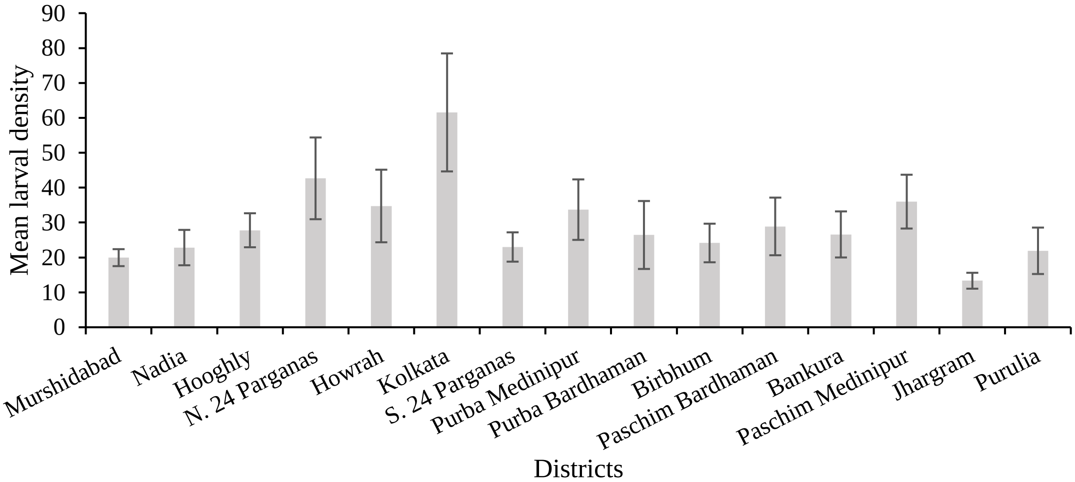

Figure 3. Analysis of district wise larval density of *Aedes* sp. (one way ANOVA analysis). Values are mean± standard error of locations

towns of West Bengal (e.g. Burdwan, Bally, Behrampur, Behala, Barasat, Midnapur, Purulia, Durgapur in addition to Kolkata) and their suburbs where coexistence of both the species in high abundance was noticed and sometime in the same habitat container. According to this study it also appears that the rural areas of this region are invaded solely by *A. albopictus* and mostly in low abundance with exceptions at few rural locations of Howrah, North 24 parganas and South 24 Parganas where larval density was high.

The results of this survey support the existing knowledge on *Aede*s distribution in India. *A. albopictus* was first identified by Skuse in 1894 in Calcutta (now Kolkata), thus being the indigenous species in this region it is found to be widely distributed in both urban and rural locations. While *A. aegypti* larvae, known to be adapted to urban environment were isolated only from towns and their peripheries. In our study both species were been found in all the districts except for Birbhum and Bankura where *A. aegypti* larva was absent. It is possible that involvement of more locations in those two districts in future could show presence of the species as well. This is one of the few studies where *Aedes* spp. was isolated and identified from rural locations of Bengal. This observation is pertinent given that dengue cases have been reported from many rural areas of West Bengal in recent years. Presence of *A. albopictus* in rural locations surveyed suggests it to be the “maintenance vector of dengue” in these areas similar to other observation [37]. Although presence of both *A. aegypti* and *A. albopictus* in rural areas of states of Tamil Nadu and Punjab, India was reported as well in most cases *A. aegypti* was identified in semi-urbanised dense regions of the villages [38,39]. The abundance of *A. aegypti* in urban areas with a high human population is due to domestication of the species, its preference towards indoor manmade habitat for breeding, and females mainly feeding on human blood [7].

Habitat characteristics and Larval survey

Similar to previous observations in other countries [40, 41], we noticed that the positive breeding habitats for *A. albopictus* were outdoors but for *A. aegypti*, larvae were identified in both outdoor and indoor containers. Since the breeding habitats of the two species are somewhat different and both exist in urban areas, the control for dengue transmission should be targeted to both types of habitats and not restricted to indoor settings.

The mean larval density calculated (Mean ± SE) was 31.99 ± 2.74. Two tailed t-test among the group means assuming unequal variance reveals that the density of the *A. albopictus* larvae in the rural (9.70 ± 1.12) and urban (36.61 ± 3.59) areas differ significantly (t = -7.16, df = 108, $p < 0.05$) though in urban locations there was no significant difference between the larval density of the two species (Table 1). This finding supports earlier reports by several other researchers from different locations [35] that *Aedes* mosquitoes are present in higher density in urban areas than in rural areas. However, one-way ANOVA analysis (Figure 3) shows no significant difference among the district wise larval densities (F = 1.396, df = 14,180, $p > 0.05$.

Types of breeding habitat varied amongst rural and urban location (Figure 4). In the urban areas, rubber tires and plastic containers for water storage were most densely populated with the larvae, whereas in rural areas, more prominently, earthen pots and tree holes had larvae (Table 2).

Outdoor habitats were preferred by both species but *A. aegypti* larvae were found in several indoor habitat (flower vases, refrigerator drain pan etc.) water as well. Two tailed t-test among the group means assuming unequal variance reveals that the mean container index (Mean ± SE) in the rural areas (2.67±0.45) is significantly lower than in urban areas (35.13±1.72), (t= -18.24, df=280, p<0.05) (Table 1).

Larval density, Stegomyia indices and human population in combination are known to be the de-

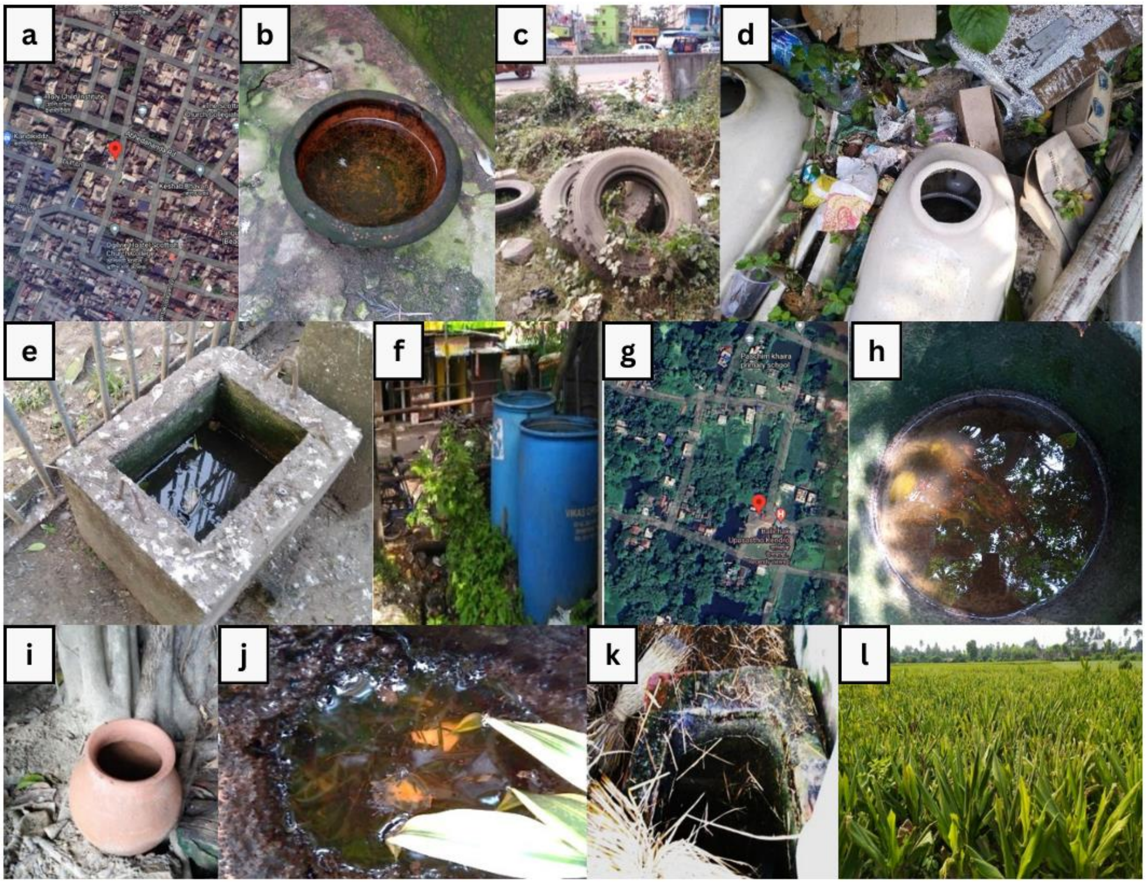

Figure 4. Photograph of one example location (with co-ordinate from google map) from each in urban area(a-b) and rural area (g-h) positive for *Aedes* mosquito breeding habitats inspected AND photographs showing examples of some breeding habitat containers in urban area: (c) tire, (d) discarded sanitary pot, (e) cement water storage, (f) plastic drums AND in rural area: (i) earthen pot, (j) tree stump, (k) earthen storage, (l) paddy stump.

Table 2. Different container characteristics in rural and urban locations and their relative distribution (%). [only mosquito-positive containers are included]

Type of container/habitat inspected	% of *Aedes* larvae positive containers	
	Rural (*n1=65)	Urban (*n2=1475)
Earthen pots/tubs	70	8
Tree holes	10	0
Cow shades and house backyard	6	0
Plastic containers	2	42
Coconut shell	5	1
Farmland irrigation	7	0
Rubber tire	0	26
Flower vases	0	2
Refrigerator drain pan	0	2
Cemented tank	0	8
Discarded porcelain/ sanitary pot	0	2
Metal containers	0	5
Private drains	0	2
Styrofoam container/ flex banners	0	2
Outdoor	**99**	**93**
Indoor	**01**	**07**

*Total number of containers found positive for larvae in rural and urban locations are(n1=65) and (n2=1475) respectively

terminants to identify potential locations for dengue occurrence [18]. The mean larval density calculated for *Aedes* population is 3.8 times more in urban locations as compared to the rural locations surveyed. The data on larval density of individual locations showed the existence of several urban and few rural locations with significantly high density of larval population. We also observed lower larval density (8 ± 5.23 or below) in locations of planned urban areas (cleaner municipalities of some districts) with proper dengue awareness as compared to high larval density (60 ± 3.72 or more) in areas with poor sanitation and lack of awareness, as in congested slums of Kolkata and unplanned, high-human populated suburbs of N.24 parganas & Howrah districts. In Howrah, South 24 Parganas, and North 24 parganas, some sporadic regions very close to rural areas were identified where improper disposal of manmade waste products provided favourable breeding habitats for Aedes, and these locations showed a high density of larvae. Surrounding rural areas could be at risk of dengue because of such dumping grounds). The variation of the physiographic conditions of this region doesn't seem to have any significant influence on larval density. The mean larval density (Mean ± SD) for the Western Plateau, Rarh, and Gangetic Delta plain regions were (28.81 ± 24.19), (26.67 ± 23.28) and (35.39 ± 45.66), respectively. One-way ANOVA analysis shows no significant difference among the region-wise larval densities ($F = 1.13$, df=2, 192, $p > 0.05$).

It is recommended that in the future, more locations should be included, and more effective determinants of dengue transmission, like house index and Breteau index [19], could thus be calculated to detect locations as possible sources of dengue outbreaks. Another interesting finding in this study was the presence of a moderate density of larvae (41±6.71) of A. aegypti in locations of Purulia district in the comparatively arid western plateau and highland of West Bengal, where the minimum temperature in winter drops down to 8^{o}C or below. *A. aegypti* is known to exist in warm weather with temperatures higher than 10^{o}C [42,43]. In light of a recent discovery that temperature triggers the transcription of multiple genes in *A. aegypti* [44], this finding will likely spur future researchers to investigate the nature of the species, its mode of survival, and propagation in this region. This finding could otherwise be explained by the phenomenon of 'global warming' that is resulting in climate change in this area, like elsewhere. Furthermore, in some locations of Howrah, N. 24 Parganas and Kolkata larvae of both species were present in the same habitat container. This finding confirms the co-existence of the two species sharing the same breeding habitat. Such co-existence could have a positive impact on the evolution of the species over the displacement of one species with the other [45,46].

Conclusion

In summary, our survey provides a more elaborate distribution pattern of the two *Aedes* species known to be prevalent in West Bengal. *Aedes albopictus* seemed to be the endemic species and is widely spread throughout the southern districts of West Bengal, whereas *Aedes aegypti* invaded this area later and is still in the process of expanding its territory either co-existing or competing with *A. albopictus*. Climate change, human growth, rapid unplanned urbanization, lack of awareness amongst common people, and poor control strategies are probably accelerating this process of expansion of the vectors into new locations. The abundance and density of the larvae observed could act as a marker for dengue risk. This pilot study thus would pave the way for further work on the vectors and may help establish advanced techniques of entomological surveillance that, in turn, would implement strategies to fight against disease in this specific region and beyond.

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