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A preliminary investigation on the container breeding mosquito *Aedes* in a non-endemic municipal city of West Bengal, India

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Abstract
The *Aedes* mosquitoes are the vectors of some deadly diseases over a major part of the World. Elimination of *Aedes* breeding sites is the key to keeping vector density under control and to do this analysis of its breeding sites in any area is a prerequisite. This study was directed to collect baseline data on the potential breeding sites of *Aedes* larvae at the Gushkara municipal area of Purba Bardhaman district in West Bengal, India. *Aedes* mosquitoes are known as container breeders. The breeding data was collected from the containers and small water accumulations having the potential as breeding sites. The House Index (HI), Container index (CI), and Breteau index (BI) of *Aedes* in the area were assessed as 3.47, 4.13, and 4.51 respectively which was lower than many endemic areas. The most potential breeding containers were discarded plastic and earthen pots. The density of house-frequenting adult *Aedes* was very low. Although the area is primarily non-endemic and the assessed indices are not alarming, the presence of potential breeding grounds in the area should not be ignored in the increasing *Aedes*-transmitted diseases like dengue incidences in the state.

Keywords: *Aedes*, larvae, breeding, containers, entomological indices, house index (HI), container index (CI), Breteau index (BI), pupal index (PI), breeding preference ratio (BPR), Gushkara

Introduction

The *Aedes* mosquitoes are the transmitters of some dreadful diseases. It is reported to be the potential vector of more than 50 medically significant arboviruses including Dengue, Chikungunya, Yellow fever, Zika, etc. over a major part of the World [1]. Globally there was an over eight folds increase in the reported dengue cases in the last two decades. An estimated 3.9 billion people in 129 countries are at risk of dengue infection including a 70% burden in the Asia region. Globally the highest number of 5.2 million dengue infections was reported in 2019 [2]. During 2021 there were 1,93,245 (346 deaths) dengue cases in India including 8,264 (7 deaths) in West Bengal [3]. Chikungunya has been identified in 110 countries with reported outbreaks in some countries in the last few decades [4]. During 2021 there were 1,19,070 suspected chikungunya cases (11,890 confirmed) in India including 154 (20 confirmed) in West Bengal [5]. Worldwide, each year an estimated 200,000 people suffer from yellow fever with about 30,000 deaths. Most of the incidence occurs in Africa and although a few cases reported, the report of any major outbreak of yellow fever from India is scanty [6]. Historically Zika infection was confined to very narrow regions but in the last two decades, it spreads to 89 countries with few epidemic outbreaks. Presently the incidence of the disease declined but low-level transmission reported in several countries including India [7]. *Aedes* is a large genus of mosquitoes with 940 species under 79 subgenera [8]. Few species of *Aedes* were reported as disease transmitters but the main vectorial role is played by two widely distributed species *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus*. *Ae. aegypti* also referred to as the 'yellow fever mosquito' originates and exists in Africa as a feral species, which later adapted to a container breeding peridomestic environment and spread rapidly in the rest of the world during the last few centuries. Today, this species persists in most countries of tropical, subtropical, and temperate regions. *Ae. albopictus* also referred to as the 'Asian Tiger Mosquito' is believed to be originated in South-East Asia, native to warm-humid tropical and subtropical countries. But this species is also reportedly adapting to cooler and temperate climates. Globally, enhanced transport systems, man-to-man contact, rapid urbanization, etc. play a key role in getting entrenchment of these mosquitoes in both rural and urban areas. In most parts of the world, *Ae. aegypti* is the principal vector of many arboviruses like dengue, while *Ae. albopictus* is recognized as an important secondary vector [9].

In many districts of West Bengal in India, the prevalence of dengue and chikungunya increased intensely, and these two *Aedes* species are recognized as the causative vectors behind them [10]. These vector-borne diseases can be controlled by controlling the vector population and the best practice to do this is to kill them in larval conditions using environment-friendly measures [11, 12]. Public perception of vector-borne diseases is often not up to the mark in many areas [13, 15].

Information on the adult population and breeding places of the vectors are necessary to apply effective control measures in any area. Several works have been conducted in different areas of West Bengal to obtain data on the adult mosquito population [16, 19], their vectorial role [10, 20, 21], and their breeding places [22, 23].

Aedes is known for its container-breeding nature and prefers clean water accumulated in artificial containers such as water tanks, flower pots and vases, buckets, discarded tyres, plastic and tin containers, tree holes and stumps, coconut shells, other discarded containers, and many more, which may differ from area to area. The present study was designed to collect primary data on the potential breeding sites of *Aedes* mosquitoes in the Gushkara municipal area of Purba Bardhaman, West Bengal, India.

Materials and methods

Study area

Gushkara is a municipal area in Purba Bardhaman district in West Bengal, India (23.50°N - 87.75°E) with a population of about 36 thousand. In addition, people from different areas visit this area for several purposes, and an ample amount of garbage is produced every day. Various waterbodies with different levels of organic pollution are present in the area [24]. The tropical climatic condition with sufficient rainfall during monsoon may increase the potential breeding containers.

Sampling and identification

Water-accumulated containers and any other small water accumulations having the potential of *Aedes* breeding in and around the households were inspected for immature mosquitoes during the year 2018 following standard methods [9, 25, 26]. Indoor-resting adults were also collected employing 16 man-hours each season using standard methods [27]. Immatures were reared to the adult stage and identified using standard keys [28, 29].

Statistical analysis

World Health Organization (WHO) recommended larval indices such as House or Premises Index (HI), Container Index (CI), Breteau Index (BI), Pupal Index (PI), and Breeding Preference Ratio (BPR) were worked out [9].

$$HI = \frac{\text{Number of Houses Positive for Larvae and/or Pupae}}{\text{Number of Houses Inspected}} \times 100$$

$$CI = \frac{\text{Number of Containers Positive for Larvae and/or Pupae}}{\text{Number of Containers Inspected}} \times 100$$

$$BI = \frac{\text{Number of Containers Positive for Larvae and/or Pupae}}{\text{Number of Houses Inspected}} \times 100$$

$$PI = \frac{\text{Number of Pupae Collected}}{\text{Number of Houses Inspected}} \times 100$$

$$BPR = \frac{\text{\% of Any Particular Container Among All Containers Inspected}}{\text{\% of That Container Positive Among All Positive Container}}$$

Results and discussion

A total of 173 immatures (larvae and pupae) were collected from the containers in the inspection of which 94.80% was *Aedes*. The dominating species of *Aedes* immatures was identified as *Ae. albopictus* (95.73%) from the area and the other species was *Ae. aegypti*. All types of containers having the potential to be a breeding site for the *Aedes* were inspected of which coconut shells, earthen pots, large plastic containers, discarded small plastic containers, metal containers & scraps, discarded tyres, and water tanks (overhead & ground) were found to be the potent ones.

In the rainy season with the increase of water-filled containers due to rainwater the HI, CI, and BI were higher (6.25, 5.45, and 9.38 respectively). Deficiency of water-filled sources in winter months these indices turn out to be lowest (1.04, 2.08, and 1.04 respectively). In the study overall HI, CI, and BI were calculated as 3.47, 4.13, and 4.51 respectively (Table 1). Pupae in the containers were encountered during the monsoon only and PI was calculated as 10.42 during that period. 70.12%, 23.78%, and 6.10% of immature *Aedes* were collected during the rainy, summer, and winter seasons respectively ($P < 0.05$).

The BPR was observed to be highest in discarded small plastic containers (0.058) followed by earthen pots (0.039) and then the other potent containers (Fig. 1).

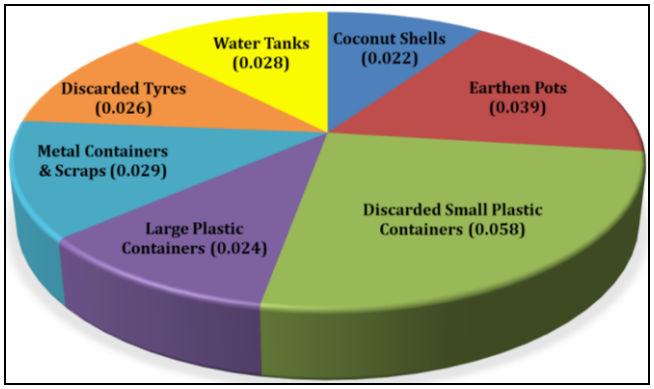

Fig 1: Breeding Preference Ratio (BPR) of *Aedes*

All the adult *Aedes* caught during the study were *Ae. albopictus* with significant seasonal variations ($P < 0.05$). 54.90%, 33.33%, and 11.76% of the adults were caught during the rainy, summer, and winter seasons respectively. The corresponding man-hour density (MHD) in the three seasons were 1.75, 1.06, and 0.38 respectively (Table 1).

Table 1: Seasonal variation in larval and adult entomological indices

Season	Immatures			Adults	
	HI	CI	BI	%	MHD
Summer	3.13	2.94	3.13	33.33	1.06
Rainy	6.25	5.45	9.38	54.90	1.75
Winter	1.04	2.08	1.04	11.76	0.38
Overall	3.47	4.13	4.51	100	1.06

Epidemic management of *Aedes*-transmitted diseases like dengue largely relies on entomologic surveillance based on different larval indices. Three levels of risk for dengue transmission were suggested by the Pan American Health Organization as low, medium, and high when HI is <0.1%, 0.1-5%, and >5% respectively [30]. But in Singapore, dengue outbreaks occurred when HI was <1% [31], whereas, in Fortaleza of Brazil, there are no outbreaks evidenced when HI was <1% [32]. In Delhi-NCT of India, the highest dengue infections were reported in late monsoon when HI, CI, and BI were 15.13, 20.96, and 47.0 respectively [33]. In dengue outbreak areas of Cheras district in Kuala Lumpur HI, CI, and BI ranges from 0.0-13.33, 0.0-13.0, and 3.57-19.05 in different localities [34]. In Purba Medinipur of West Bengal in India, in the rural areas with a suspected dengue outbreak, the HI, CI, and BI were 8.0, 13.1, and 9.5 respectively [35].

Studies suggest that it is very difficult to standardize the threshold values of the vector/entomological indices for all the areas [36]. As these indices may get influenced by many regional factors such as topography, environment, vegetation, characteristics of the vector and the human population, perception of the community, etc. Moreover, the usefulness of these indices is often argued as they have some serious shortcomings and maybe that's why critical thresholds of these indices have never been developed for dengue transmission [37]. However, threshold values of larval indices for yellow fever transmission have been suggested many decades ago (Table 2) [38]. Many epidemiologists and organizations prefer to use those

threshold values used for yellow fever in case of dengue epidemic management also due to the resemblance between yellow fever and dengue epidemiology, transmission, and vectors [37, 39, 40].

When the indices are compared with the risk levels (Table 2) suggested by WHO (1971) [38] with the present study, it is observed that all the indices remain within 'low risk' level in the summer and rainy seasons but those indices reaching the 'moderate risk' in the rainy season. Overall CI in the area was observed at a 'moderate risk' level but HI and BI were at 'low risk'. The density of the adult population was low in the study area. Discarded plastic and earthen containers are the preferred breeding habitats of the *Aedes* in the area.

Table 2: Entomological indices and risk of yellow fever transmission (WHO, 1971) [38]

Risk	HI	CI	BI
Low Risk	< 4	< 3	< 5
Moderate Risk	4 - < 35	3 - < 20	5 - < 50
High Risk	≥ 35	≥ 20	≥ 50

Conclusions

Routine vector surveillance is recommended to assess the risk of vector-borne disease outbreaks and formulation of vector management strategies [9]. Overall larval indices of *Aedes* in the Gushkara area show a lower risk level of *Aedes*-transmitted diseases throughout the year except in the monsoon months risk level increases a little. A positive correlation between the entomological indices and rainfall was noticed as also reported in other studies [33]. Indices were lower in comparison to many dengue-outbreak areas [33, 35, 40]. Information of any major outbreak of *Aedes*-transmitted diseases was also scanty. But in view of the rapidly increasing incidence of dengue and chikungunya throughout the world including the West Bengal state, evidence of the low index values triggering diseases in many areas, with the availability of the breeding habitats, and the potency of the *Aedes* in the present study area, regular surveillance is suggested.

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