Entomological Survey for Identification of Aedes Larval Breeding Sites and Their Distribution in Chattogram, Bangladesh

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Research

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Abstract

Background: Studying the characteristics of *Aedes* mosquito habitats is essential to control the mosquito population. The objective of this study was to identify the breeding sites of *Aedes* larvae and their distribution in the Chattogram. We conducted an entomological survey in 12 different sub-district (Thana) under Chattogram city, Bangladesh, during the late monsoon (August to November) 2019. The presence of different wet containers along with their characteristics and the existence of immature mosquitoes were recorded in field survey data-form. Larvae and/or pupae were collected and brought to the laboratory for identification.

Results: We estimated the overall house index, container index, and the Breteau index and performed multiple logistic regression analyses to identify habitats more likely to be positive for *Aedes* larvae/pupae. Out of a total of 704 wet containers of 37 different types from 216 properties where 52 (7.39%) containers were positive for *Aedes* larvae or pupae. Tire, plastic buckets, plastic drums, and coconut shells were the most prevalent container types. The plastic group possessed highest container productivity (n=50) whereas vehicle and machinery group was the highest efficient (1.83). Among the total positive properties, 8% were infested with *Aedes aegypti*, 2% were *Aedes albopictus* and 1% contains both species *Ae. aegypti* and *A. albopictus*. The overall house index was 17.35%, container index was 7% and the Breteau index was 24.49. Containers in multistoried House had significantly lower positivity in compare to independent house. Binary logistic regression represented that containers having shade were 6.7 times more likely to be positive than the containers without shade (p< 0.01).

Conclusions: These findings might assist the authorities to identify the properties, containers, and geographical areas with different degrees of risk for mosquito control interventions to prevent dengue and other *Aedes*-borne diseases transmissions.

Background

*Aedes* mosquitoes (Diptera: Culicidae) are a highly efficient vector of various arboviruses including dengue, chikungunya, zika, etc., around the globe. Dengue fever is an important mosquito-borne disease (MBD) that pose risk for the people of more than 128 countries of the world [1]. *Aedes aegypti* is the primary vector of the dengue virus (DENV) which is found in urban areas due to its anthropophilic blood-feeding behaviour. *Aedes albopictus* belongs to the same subgenus (*Stegomyia*) and is considered as a secondary vector of DENV. They found mostly in rural areas but also in peri-domestic sites of urban areas like parks and green corridors interspersing housing estates. Both of these species were first recorded in Bangladesh in 1952 [2]. Since 2000, Dengue fever (DF) cases have been reported every year in all major cities in Bangladesh [3]. The outbreak of Chikungunya fever took place in 2008 and the Zika virus was also introduced in the country in 2016 [4, 5]. During 2019, Bangladesh recorded its largest dengue virus outbreak with 101,354 cases, more than double the cases in the last 20 years [6]. The *Aedes* spp. mostly breed in artificial water-holding containers, but have been reported in natural containers as well [7]. Vector control remains the center of dengue prevention options, which can be done by limiting the transmission potential by reducing the emergence of adult mosquitoes. This could achieve by targeting the aquatic habitats of the immature stages of *Ae. aegypti* through source reduction or biological and/or biocidal treatment [8, 9]. The productivity of a container type depends on a range of factors, such as size and shape, the purpose of use, their locations (such as whether they are indoors, outdoors, under vegetation, etc.), method of filling (passively/actively), the material with which the container is made (plastic, metal, cement/clay, etc.), temperature, availability of food, and competition among co-species [10, 11].

Various entomological indices are used to measure dengue vector infestation in and around infrastructures, such as homes, buildings, etc. [12]. Examples of such indices are the different *Stegomyia* indices- house index (HI, the proportion of *Aedes* positive houses) and container index (CI, the proportion of *Aedes* positive containers), and y the Breteau index (BI, the number of *Aedes* positive containers per 100 houses) [13, 14]. As there is a lack of a sensitive vector surveillance tool to estimate the vector density in the outbreak areas, the vector abundances for both *Ae. aegypti* and *Ae. albopictus* are still expressed as House index, Breteau index, and Container index.

A few studies on *Aedes* mosquito abundance and breeding preference was conducted in the capital city Dhaka, Bangladesh but data on other big cities like Chattogram, the 2nd popular city in the country are still absent. Therefore, during the late monsoon (August to November) of 2019, we conducted a comprehensive entomological survey in Chattogram. The expansion of
residential areas to natural ecosystems, continuous changes in biodiversity and growing industrial areas are alarming in Chattogram which might promote the human-vector contact and the transmission of arboviruses to humans [15]. The objective was to identify the breeding sites of *Aedes* larvae and their distribution in the Chattogram metropolitan area.

**Methods**

This entomological survey was conducted in late monsoon in 2019 between August-November, the time when dengue incidence was high in the study area as well as throughout the Bangladesh [6]. The study location was the Chattogram city of Bangladesh. Chattogram is the second largest city of Bangladesh and an area poorly studied in terms of mosquito survey. A total of 12 sub-districts (Thana) under the Chattogram City Corporation (CCC) area were chosen purposively for this survey. A Thana is defined as the small administrative boundaries within the metropolitan area.

**Entomological Survey:**

An entomological survey for DENV was designed to detect immature stages of the *Aedes aegypti* (L.) (Diptera: Culicidae). Our survey targeted all groups of juveniles (1st – 4th instar larvae and pupae) by inspecting of all accessible water-holding containers in public and private areas, to identify the most productive and efficient container types for these species. Samples were taken by pipetting, dipping, or netting (WHO 1997) in small plastic jars with water. Each sample jar was labeled with the unique identification number, date, location, number of collected larvae/pupae. Breeding habitats of the collected mosquito species were recorded in a pre-defined survey data-sheet during the sample collection. Different indices were calculated to document the primary breeding source and density of the *Aedes* mosquito. A container was recorded as positive for *Aedes* if one or more juvenile *Ae. aegypti* or *Ae. albopictus* was found in the given type of container and were distinguished from those with no juveniles (negative)

**Selection Of Properties**

18 different locations were surveyed, including government-owned properties. The properties were purposively selected from each Thana and were categorized into nine classes, depending on the possession of living properties, working stations, and other public gathering places, as shown in Table 1.

| Category                | Number | Definition                                                                 |
|-------------------------|--------|-----------------------------------------------------------------------------|
| Independent House       | 4      | These were brick-built single-family homes, either single floor or duplex with surrounding garden. |
| Multistoried House      | 4      | These were brick-built apartment houses having two or more floors. More than one family lives in these houses. |
| Slum                    | 4      | Slum houses are usually made of bamboo and tin, or even by mud walls. These are inadequate infrastructure with congested tenements. |
| Construction site       | 1      | independent or multi-storied buildings in the construction phase              |
| Police station          | 1      | The local police station area in each Thana. This is usually an area of single building with open place and garden. |
| Educational institution | 1      | Any properties like schools, colleges, universities, or any others used for educational purposes, comprising small or big areas. |
| Hospital                | 1      | Government or private hospitals, clinics diagnostic centers                  |
| Open place/park         | 1      | Any sort of open spaces like parks, roadside places, or playgrounds without any establishment |
| Bus stand/garage        | 1      | Small and large bus stands, garages, or fuel station                         |

**Classification Of Containers Found As Larval Development Sites**
The containers were divided into two broad groups based on their purposes: controllable and disposable ones. Controllable containers were household containers that could be manipulated by man to avoid mosquito larval breeding which included concrete tanks, metal drums, flower pots, aluminum tanks, small buckets, and other plastic containers used to carry or store water. Disposable containers were those that are not used in households, are abandoned or stored in backyards having the potential as breeding sites in the rainy season. Examples of disposable containers include tires, cans, tubs, etc.

Identification Of Mosquitoes

After collection, the larvae and/or pupae were brought to the pathology and parasitology laboratory. Larvae were identified under microscopes in the laboratory. Pupae and the rest of the larvae were reared in rearing trays for the identification of adult mosquito. Species identification was completed using standard identification keys as described by [16]. The laboratory findings were recorded in the corresponding survey sheet.

Determine The Key Containers

All wet containers were divided into 6 categories based on the materials they were composed of, or the purposes they served in, namely, plastic receptacles, tin & metal receptacles, cement & clay receptacles, natural receptacles, vehicles & machinery parts, and other receptacles. The role of these groups of containers regarding the production of Aedes mosquito was estimated by the value of container productivity and container efficiency mentioned by [17]

PC = Positive containers

CP = Container productivity (no. of immature x 100/all immature)

CE = Container efficiency (productivity/prevalence of container)

Prevalence of container = no. of wet containers x 100/all containers

Estimate Different Indices

The number of different wet containers, the percentage of positive containers category, and finally, the percentage of Aedes larvae in each container category were calculated to determine the different Stegomyia indices as follows [18]

Container index: the percentage of water holding containers infested with larvae and/or pupae

House Index: the percentage of house infested with larvae and/or pupae

Pupae index: number of pupae per 100 households

Breteau index: the number of positive containers per 100 houses inspected.

Statistical analysis

We performed two different statistical analyses in this research i) zero-inflated negative binomial regression to find the factors associated with the number of immature Aedes positive mosquito per container, ii) binary logistic regression to find the odds of the background characteristics causing the containers to be Aedes positive. The exponential of the parameter of the logistic regression was the odds of the category of the background characteristics being Aedes positive of water-holding containers. The exponential of zero-inflated negative binomial regression was the ratio of the average number of immature Aedes positive mosquito among the groups of background characteristics. Data analysis was conducted in R version 3.5.2 and the binary logistic regression model was performed in SPSS version 25.

Results

A total of 704 wet containers were identified in around 216 surveyed properties, with a mean of 3.3 containers per property. Of these, 204 (29%) were controllable and 500 (71%) were disposable ones. More containers were identified on privately owned
properties (N = 531, 75%) then government (N = 173, 25%) owned properties. We collected 573 *Aedes* juveniles, including 101 pupae. The number of *Ae. aegypti* (n = 371) was higher than the number of *Ae. Albopictus* (202). We calculated the house index as 17.35%, container index 7%, and the Breteau index 24.49. The distribution of the background characteristics of the entomological survey is summarized in Table 2.
Table 2
General distribution of the background characteristics of wet containers in Chattogram, Bangladesh.

| Variables | Category                  | Frequency | Percentage | \textit{Aedes} positive container (%) |
|-----------|---------------------------|-----------|------------|--------------------------------------|
| Container function | Controllable              | 204       | 29         | 15(31)                               |
|            | Deposable                 | 500       | 71         | 34(69)                               |
| Ownership  | Government                | 173       | 25         | 16 (32.65)                           |
|            | Private                   | 531       | 75         | 33 (67.35)                           |
| Vegetation | No                        | 401       | 58         | 30(61)                               |
|            | Yes                       | 297       | 42         | 19(39)                               |
| Shade      | No                        | 129       | 18         | 2(4)                                 |
|            | Yes                       | 572       | 82         | 47(96)                               |
| Property Category | Independent House         | 157       | 22         | 16(33)                               |
|            | Multistoried House        | 141       | 20         | 4(8)                                 |
|            | Slum                      | 134       | 19         | 10(20)                               |
|            | Police Station            | 67        | 10         | 3(6)                                 |
|            | Hospital                  | 48        | 7          | 0(0)                                 |
|            | Bus stand                 | 51        | 7          | 4(8)                                 |
|            | Educational Institutions   | 37        | 5          | 5(10)                                |
|            | Open place/park           | 49        | 7          | 6(13)                                |
|            | Construction              | 20        | 3          | 1(2)                                 |
| Container Category | Plastic Reservoir         | 144       | 37.1       | 127(17)                              |
|            | Metal/tin/aluminum reservoir | 23     | 5.9       | 21(2)                                |
|            | Cement/clay/ceramic reservoir | 29     | 7.5       | 25(4)                                |
|            | Natural and plant material | 96        | 24.7       | 90(6)                                |
|            | Vehicles and machinery items | 56    | 14.4       | 45(11)                               |
|            | Others                    | 40        | 10.3       | 38(2)                                |
| Geographic Location | Khulshi                   | 109       | 15         | 8(16)                                |
|            | Pahartali                 | 70        | 10         | 13(27)                               |
|            | Bakalia                   | 41        | 6          | 2(4)                                 |
|            | Kotowalli                 | 62        | 9          | 1(2)                                 |
|            | Panchlais                 | 52        | 7          | 11(23)                               |
|            | Double Mourning           | 55        | 8          | 5(10)                                |
|            | Halishahar                | 22        | 3          | 3(6)                                 |
|            | Bandor                    | 43        | 6          | 1(2)                                 |
|            | Chandgawn                 | 91        | 13         | 1(2)                                 |
|            | EPZ                       | 51        | 7          | 1(2)                                 |
| Variables | Category               | Frequency | Percentage | \(Aedes\) positive container (%) |
|-----------|------------------------|-----------|------------|----------------------------------|
| Akbarshah | 69                     | 10        | 3(6)       |
| Bayzid    | 39                     | 6         | 0(0)       |

**Distribution of positive containers and juvenile \(Aedes\) spp.**

We examined 704 wet containers of 37 varieties, where 49 (6.9%) wet containers were positive for immature \(Aedes\) mosquitoes. Among positive containers, 31% (n = 15) were controllable (plastic bucket, plastic drums, plastic bag and earthen jar) and 69% (n = 34) were the disposable (containers like tires, coconut shell, cock sheet, mineral water jar and bamboo hole). 32.65% (n = 16) positive containers were found in government-owned properties and the rest 67.35% (n = 33) were in private properties. Among positives, 96% (n = 47) of containers were under shades and 39% (n = 19) had the presence of vegetation in and around the container. Other than residential areas, different open places or parks contained 13% (n = 6), and educational institutions had 10% (n = 5) of the positive containers. Figure 1 shows the distribution of positive containers in different Thanas.

A total of 216 properties from 12 Thanas under the Chattogram City Corporation (CCC) area were visited, where 16.2% (n = 35) properties were positive for immature \(Aedes\) mosquito. Among the total positive properties, 7.2% (n = 28) were infested with \(Aedes\) aegypti and 2.3% (n = 9) were \(Aedes\) albopictus. Moreover, \(Ae.\) aegypti co-existed with \(A.\) albopictus in 1.3% (n = 5) properties. Among individual containers, tires 16.33% (n = 8) were revealed as the most productive container for the \(Aedes\) larval breeding, followed by plastic buckets 14.29% (n = 7), coconut shells 12.24% (n = 6), plastic drums 12.24% (n = 6), and flower tub and tray 8.16% (n = 4) (Fig. 2).

**Determining The Key Containers**

According to immature \(Aedes\) production in different container categories, plastic container had most abundantly (33%) found as positive and also possess highest container productivity (50). Vehicle and machineries group was highest efficient (1.83) and 2nd most productive (30) group. Natural group contained 27% positive containers with container productivity 11. So, these 3 container categories could be considered as important for \(Aedes\) spp. mosquito breeding. The role of all container categories has shown in Table 3.
Table 3

The efficiency of different container categories for the production of Aedes larvae

| Container category       | Containers                                                                 | Frequency (%) | Positive containers (%) | Immature Aedes | CP  | CE  |
|--------------------------|----------------------------------------------------------------------------|---------------|-------------------------|----------------|-----|-----|
| Plastic receptacles      | Plastic bucket, Plastic drum, Mineral water jar, Dahi pot, Plastic bag, Water tank, Plastic bottle, Plastic mug | 215 (31)      | 16 (33)                 | 4818           | 50  | 1.62|
| Tin and metal receptacles| Metal/tin can, Metal bucket, Metal drum, Metal pan Aluminum pot           | 47 (7)        | 2 (4)                   | 345            | 3.5 | 0.53|
| Cement and clay receptacles | Cement pot, clay pot, animal bowl, glass bottle, ceramic pot, melamine bowl, earthen jar | 86 (12)       | 4 (8)                   | 295            | 3   | 0.25|
| Natural receptacles      | Coconut shell, mud hole, bamboo stamp, plant axil, tree hole, flooded floor, money plan tub, flower tub, and tray. | 181 (25)      | 13 (27)                 | 1086           | 11  | 0.43|
| Vehicles and machinery parts | Vehicle parts, tires, battery, water motor, cock sheet, air cooler, musical instrument, cement mixture | 116 (17)      | 12 (24)                 | 2934           | 30  | 1.83|
| Others receptacles       | Unused sheet, plastic sheet, discarded food wrapper, broken toilet parts   | 59 (8)        | 2 (4)                   | 275            | 3   | 0.34|

CP = Container productivity (no. of immature x 100/all immature)
CE = Container efficiency (productivity/prevalence of container)
Prevalence of container = no. of wet containers x 100/all containers

Zero-inflated negative binomial regression (Table 4) revealed that the number of immature Aedes mosquito produced per container had a significant association with the property category and shade (p < 0.01). Binary logistic regression (table 5) elucidated that containers having shade had 6.7 times more likely to be positive than the containers without shade (p < 0.05). Containers in multistoried house had significantly lower positivity in compare to independent house. Besides, Kotowali and Bandar thana had produced significantly lower number of positive containers (p < 0.05) as their odds found below one

Table 4

Zero Inflated Negative Binomial Regression of Aedes positive larvae/pupae with the background Characteristics of water-holding containers, Chattogram, Bangladesh, 2019

| Predictors                  | Estimated | Standard error | Odds Ratio | Confidence Interval at 95% level |
|-----------------------------|-----------|----------------|------------|---------------------------------|
| Vegetation (Yes)            | 0.2212    | 0.2506         | 1.247573   | 0.7633177 2.0389762             |
| Function (Disposable)       | -0.06422  | 0.39760        | 0.937799   | 0.4302001 2.0443182             |
| Container Category          | 0.13840   | 0.10919        | 1.148435   | 0.9271832 1.4224860             |
| Property Category (Mult. house) | 1.49129   | 0.64479        | 4.442823   | 1.255475 15.721946 **           |
| Location (Kotowali)         | 2.02446   | 1.15621        | 7.572021   | 0.7853205 73.0097627*           |
| Property type (private)     | -0.02158  | 0.57382        | 0.978651   | 0.3178280 3.0134419             |
| Shade (Yes)                 | -1.87277  | 0.77507        | 0.153697   | 0.0336452 0.7021209 **          |

Significance codes: 0.01 ** 0.05 *

Table 5: Binary Logistic Regression to identify factor associated with presence of Aedes sp larvae and/or pupae.
### Table 1: Predictors and Their Estimated Effects

| Predictors                              | Estimated | Standard error | Odds Ratio | Confidence interval at 95% |
|-----------------------------------------|-----------|----------------|------------|---------------------------|
| Function (Controllable)                 | 1         |                | 1          |                           |
| Function (Disposable)                   | 0.091     | 0.431          | 1.095      | 0.471                     | 2.547       |
| vegetation (No)                         |           |                | 1          |                           |
| vegetation (Yes)                        | 0.685     | 0.42           | 1.983      | 0.87                      | 4.521       |
| Property Type (Gov.)                    |           |                | 1          |                           |
| Property type (Private)                 | 0.15      | 0.695          | 1.162      | 0.297                     | 4.539       |
| Shade (No)                              |           |                | 1          |                           |
| Shade (Yes)                             | 1.902     | 0.808          | 6.701      | 1.374                     | 32.675*     |
| Property category (Independent House)   |           |                | 1          |                           |
| Property category (Multistoried House)  | -1.246    | 0.605          | 0.288      | 0.088                     | 0.942*      |
| Property category (Slum)                | -0.381    | 0.526          | 0.683      | 0.244                     | 1.915       |
| Property category (Police Stations)     | 0.358     | 1.104          | 1.431      | 0.164                     | 12.461      |
| Property category (Hospitals)           | -19.407   | 7369.331       | 0          | 0                         | .           |
| Property category (Bus Stations)        | 0.03      | 0.905          | 1.031      | 0.175                     | 6.07        |
| Property category (Educational Institutions) | 0.313   | 1.033          | 1.368      | 0.181                     | 10.357      |
| Property category (Open Place)          | 0.114     | 0.819          | 1.12       | 0.225                     | 5.575       |
| Property category (Construction Site)   | -0.07     | 1.26           | 0.933      | 0.079                     | 11.018      |
| Thana (Khulshi)                         |           |                | 1          |                           |
| Thana (Pahartali)                       | 0.421     | 0.787          | 1.524      | 0.326                     | 7.127       |
| Thana (Bakalia)                         | -1.27     | 0.931          | 0.281      | 0.045                     | 1.74        |
| Thana (kotowali)                        | -2.538    | 1.176          | 0.079      | 0.008                     | 0.793*      |
| Thana (Panchlaish)                      | 0.285     | 0.657          | 1.33       | 0.367                     | 4.823       |
| Thana (Double Mouring)                  | -0.925    | 0.745          | 0.397      | 0.092                     | 1.708       |
| Thana (Halishahar)                      | -0.098    | 0.875          | 0.907      | 0.163                     | 5.041       |
| Thana (Bondor)                          | -2.45     | 1.182          | 0.086      | 0.008                     | 0.876*      |
| Thana (Chandgao)                        | -1.74     | 1.185          | 0.175      | 0.017                     | 1.791       |
| Thana (EPZ)                             | -2.281    | 1.207          | 0.102      | 0.01                      | 1.089       |
| Thana (AkbarShah)                       | -1.406    | 0.826          | 0.245      | 0.049                     | 1.237       |
| Thana (Potenga)                         | -20.22    | 6992.331       | 0          | 0                         | .           |
| Container Category (Plastic)            |           |                | 1          |                           |
| Container Category (Metal)              | -0.547    | 0.939          | 0.579      | 0.092                     | 3.649       |
| Container Category (Cement)             | -0.439    | 0.682          | 0.644      | 0.169                     | 2.452       |
| Container Category (Natural)            | -0.941    | 0.587          | 0.39       | 0.123                     | 1.234       |
| Container Category (Vehicles)           | 0.334     | 0.533          | 1.397      | 0.491                     | 3.973       |
| Container Category (Others)             | -1.724    | 0.851          | 0.178      | 0.034                     | 0.945*      |

Significance codes: 0.01 *** 0.05 *

### Discussion

We inspected a broad spectrum of wet containers from different properties in the second most populated city of Bangladesh and identified *Aedes* larvae and/or pupae. The total number of *Ae. aegypti* larvae and/or pupae were higher than the number of *Ae. Albopictus* and the record were consistent with previous studies [19, 20]. Opposite results found in Malaysia that 77% breeding containers were positive for *Aedes albopictus* and 23% for *Aedes aegypti* [21]. As the survey was conducted in city areas only, the breeding sites of *Ae. albopictus* were reached partially which might be responsible for the lower count of *Ae albopictus*. The mean number of wet containers per property was 3.3 in our study, which is comparatively lower than the mean numbers of two previous studies 4.20 found in Dhaka [7] and 7.9 found in Southern Mexico [22]. A possibility could be because people were more aware of during the devastating dengue epidemic in 2019.
The overall house index (HI), container index (CI), and Breteau index (BI) were calculated high which is supported by a similar study conducted in Dhaka earlier which stated the overall HI 14.2%, BI 24.65, and CI 5.9% [7]. All of the indices showed a high level of risk for dengue transmission. A survey in Nepal found higher CI in the post-monsoon period than monsoon [23]. A recent study in Malaysia showed maximum HI = 13.33%, BI = 13, and CI = 19.05% [21]. It has been criticized in the literature [13] that these indices are of operational value and are of limited use in assessing the transmission risk. However, these indexes are indicative of the global transmission risk at the community level.

The biggest portion of Aedes larvae containing water-holding containers were under the group of disposable containers. The estimated larval production in disposable containers in other settings were 70% and 55.4% [22]. This indicates that the proper and regular disposal of unused containers will make a great contribution to vector control programs. The common containers for Aedes breeding were plastic containers (30%) in Kuala Lumpur, Malaysia [21]. As individual container types, we found the contribution of unused tires as the highest for Aedes larval breeding along with other key containers-plastic buckets, plastic drums, coconut shells, and flower tubes and trays. Our results are consistent with the findings of two previous studies carried out in Dhaka, Bangladesh, conducted during 2011-13 [24, 25]. A study in Nepal stated discarded tires as the most preferable wet containers [23]. Another study revealed flower pots as the principal types of containers with Ae. aegypti larvae [26].

Within a community, not all houses have the same potential for larval breeding, and the identification of those houses that are likely to have high larval production is important to better direct control activities. Independent households, having disposable wet containers in shaded outdoor premise were significantly associated with household infestation of Aedes larvae [7]. Notably, the survey found more juveniles in containers of private properties compared to those on government properties, similar to the findings of a previous study which showed that more containers on privately owned properties than in government-owned properties [27] private properties also have 1.7 times greater odds (95% CI = 1.1 – 2.5) of being infested compared with containers on government properties [28]. We observed Ae. aegypti and A. albopictus co-existed in different properties which is also reported earlier in Jinghong city, China [29]. It suggested that both species should be managed during dengue epidemic season.

This study revealed that independent houses were the most infested with containers possessing Aedes larvae, followed by slums and open places/parks. The findings are concordant with the earlier studies conducted in other cities of the country which stated that household positivity rate was the highest in independent houses (18.6%), followed by slum houses (14.3%), semi-permanent houses (12.9%), and multi-storied houses (12.8%) [7]. Found of positive containers in open places/parks might impose risk of the dengue fever for city dwellers as a study in a Japan during dengue epidemic showed that more than 80% of dengue patients visited a city park where Ae. albopictus was present [30]

The observations or experimental units were the water holding container spread over the different locations in Chittagong city. We analyzed the count data of the number of immature Aedes positive larvae and/or pupae per container, with access zero accounts for non-Aedes, by a statistical model. By using the predictors of background characteristics (container function, ownership, vegetation, shade, property category, container category, and geographic location) of water-holding containers, a multiple regression model was applied [27]. To deal with access zero, we considered zero-inflated Poisson regression model [31] and zero-inflated negative binomial regression model [32] to find the association between the number of immature Aedes positive mosquitoes per container and the background characteristics of the water-holding containers. Both of the considered models are modifications of the count data regression model. We have calculated the Akaike Information Criteria (AIC) value to find the improved model between the two [33, 34]. The zero-inflated negative binomial regression model (543.82) has less AIC value than the zero-inflated Poisson regression model (693.16). Hence, the zero-inflated negative binomial regression model better fit our data.

We observed that wet containers which were under shades were more likely to have Ae. aegypti, unlike previous findings where more larvae and pupae were detected in containers without shades compared to the containers located in areas with full shades [25]. The findings may have been due to the classification of containers as partially shaded and fully shaded by the researchers. However, a recent study in Dhaka found that containers with partial shades produced 4.6 times more pupae than without any shade [24]. Containers located outdoors and proximal to vegetation were significant for producing larvae. [28]. We also found odds greater than one for containers possessing vegetation. Vegetation in and around containers might enhance mosquito
breeding by facilitating resting sites for gravid females to lay eggs and important sugar feeding resources for larvae [35]. The larval production of any species of mosquitoes is significantly affected by the presence of vegetation near the containers [25].

We examined the surroundings outside of the properties, excluding the inside. The total number of containers was also limited to some extent. A few recent studies used different container parameters like size or volume of the container, exposure to sunlight, cover status, and filling methods of the containers for evaluating container productivity. In our study, we considered exposure to sunlight/shade and vegetation status, function composing materials of containers. Previous studies suggested, the extensive vector control program by reducing breeding sites could maintain a very low Aedes house index [26]. that weekly cleaning of the water-holding containers were effective in the control of larval production [20].

**Conclusions**

We detected *Ae aegypti* in 8% of the properties, *Ae albopictus* in 2% of the properties, and the co-existence of both *Ae. aegypti* and *A. albopictus* in 1% of properties, which posed high risks of dengue transmission. We identified that the presence of shades on the breeding sites can significantly increase the risk of having *Aedes spp* mosquito larvae. Plastic containers were the most abundant and productive containers while the vehicle and machinery category was found as the most efficient container group for *Aedes* mosquito breeding. We recommend vector control through regular inspection and destruction of potential container types as a way to prevent the dengue outbreak.

**Abbreviations**

*Ae*: *Aedes*

/ : divide

Etc.: etcetera

< : less than

x : multiply

n: number

% : percentage

R: Analytic software

2nd: second

Spp.: species

SPSS: Statistical packages for social sciences

**Declarations**

**Ethics approval and consent to participate**

This study was carried out in accordance with the recommendations of the Chattogram Veterinary and Animal Sciences University’s Ethics Committee. However this study was waived for written approval and consent as it didn't involve human participants, human data or human tissues.

**Consent for publication**

The manuscript has not been submitted to more than one journal for simultaneous consideration. The manuscript has not been published previously (partly or in full). No data have been fabricated or manipulated (including images) to support our
conclusions. No data, text, or theories by others are presented as if they were the author's own.

**Availability of data and material**

Data and supporting materials will be available from the authors upon reasonable request.

**Competing interests:**

The authors declare that there is no conflict of interest.

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**Authors’ Contributions**

Conceptualization: MSR, NH. Methodology: MSR, NH. Field-work and data curation: MSR, ST, NMS. Laboratory work: MSR, Data analysis: MSR, OMF, SC. Writing - original draft: MSR, MOF, ST, NMS. Writing - review & editing: SC, MSR, NH. All authors have read and approved the manuscript.

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