**Seasonal Distribution and Container Preference Ratio of the Dengue fever vector (*Aedes aegypti,* Diptera: Culicidae) in Rawalpindi, Pakistan.**

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**ABSTRACT:**

*Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) are known vectors of dengue, chikungunya and other pathogens; however, their ecology and role in virus transmission has not been well studied in Pakistan. Here, we report on an intensive survey of potential breeding sites of *Ae. aegypti* in Rawalpindi, Punjab Province, Pakistan. The study continued for 11 months and was divided into three seasons: January to June (Pre-monsoon), July to September (Monsoon) and October-November (Post-Monsoon). Larval mosquitoes were collected from all wet containers present in and around the houses. Altogether 5,570,418, 2,930,508 and 1,507,111 water-filled containers were examined during each season, of which 2,703, 8,843 and 3,439 were found positive for *Ae. aegypti* larvae or pupae, yielding Breteau indices of 0.46, 2.92 and 1.99%, respectively. Among fourteen container types examined, the breeding preference ratio during all seasons was highest for roof-top water tanks and room evaporative coolers, followed by discarded tires and urban trash. The study concluded that increased urbanization, insufficient water supply and inefficient removal of urban trash resulted in increased numbers of non-biodegradable containers around human dwellings, thereby creating ideal breeding habitats for *Ae. aegypti*. Measures such as integrated vector management, minimization of the breeding potential of *Ae. aegypti* by water management, proper disposal of discarded tires and urban trash and health education were recommended for control of *Ae. aegypti*.

**Keywords:** Container preference ratio, *Aedes aegypti*, Dengue, Pakistan, Seasonal Distribution

**Introduction:**

In Pakistan the first epidemic of dengue fever was reported in 1994 (Khan et al. 2007) when dengue virus emerged as the most significant mosquito-borne viral disease affecting humans (Khan et al. 2010). In Pakistan, from 2006 to 2011, 40,987 cases of dengue were reported with 490 deaths (Wld. Hlth. Org. 2012).). In August 2013, a large dengue outbreak occurred in Kyber Pakhtun khaw Province affecting more than 7,000 people with 26 deaths (Khan et al. 2007). In Rawalpindi District, there were 1,100, 1,406 and 3,900 confirmed cases of dengue fever in 2013, 2014 and 2015, including seven deaths in 2015 (Zaki et al. 2016).

Pakistan, as a whole, is highly susceptible to epidemics of dengue, because the climate is dominated by a summer monsoon and the urban environments are suitable for *Aedes* mosquitoes. Infrastructure problems such as erratic water supply (that forces the population to store water in containers), and illiteracy/ low education levels with poor sanitation combine with an increasing population density to produce ideal conditions for high mosquito densities.

In Pakistan, there are 30 known species of *Aedes*, of which two species, *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse)*,* are considered as the primary and secondary vectors of dengue virus, respectively (Gubler 1989). Female *Aedes* of these two species lay their eggs in natural and artificial water-holding containers (Gubler and Kuno 1997). *Aedes aegypti* is a domestic mosquito that oviposits in containers placed close to or within houses, including traditional water tanks, fountains, bird baths, and almost any containers that hold fresh water (Taylor et al. 2008).

The present paper describes the results of an entomological survey to determine the larval indices and container preference ratios of *Ae. aegypti* in Rawalpindi, Pakistan, with the purpose of improving dengue control strategies.

**Materials and Methods**

**Study Area and Site selection**

Rawalpindi is the 4th most populous metropolitan area of Pakistan. Situated in the northern most part of [Punjab](https://en.wikipedia.org/wiki/Punjab) Province ([33°36′N 73°02′E](https://tools.wmflabs.org/geohack/geohack.php?pagename=Rawalpindi&params=33_36_N_73_02_E_type:city%283363911%29_region:PK)) has a total area of 5,286 km2. Its location is central with the capital Islamabad to its north, Punjab to its south, Kyber Pakhtun khaw Province to its west and [Azad Kashmir](https://en.wikipedia.org/wiki/Azad_Kashmir) to its east. For this reason travelers use the city as a stopping point before traveling towards northern areas. Rawalpindi features a [humid subtropical climate](https://en.wikipedia.org/wiki/Humid_subtropical_climate) with long and very hot summers, a [monsoon](https://en.wikipedia.org/wiki/Monsoon) season, and short, mild and wet winters. The average annual rainfall for year 2016 is (992 mm), most of which (500 mm) falls in the summer monsoon season of just three months (July-Sep). (http://www.pmd.gov.pk) .In summer, the record maximum temperature is 46.5 [°C](https://en.wikipedia.org/wiki/Celsius) while the winter record minimum is −3.9 [°C](https://en.wikipedia.org/wiki/Celsius). (<https://en.wikipedia.org/wiki/Rawalpindi>).

The study was based on a cross-sectional entomological survey from January to November 2016 in Rawal, Potohar and Cantonment area towns to broadly represent residential areas, parks, commercial areas, junkyards and representative urban habitats. **T**he sampling unit was the house or premise which was searched for water holding containers by two collectors. Approximately 5% of the houses or every 20th house was inspected systematically during each weekly visit. Each town was visited repeatedly until most of the houses were visited during each season. The larval collections were done by a team of 900 insect collectors working under the direction of four entomologists.

**Mosquito Collection**

All wet containers both indoors and outdoors which might harbor mosquito larvae were inspected. Bottles, flasks, tins and other small containers containing water were emptied directly into a tray (with white background) or filtered through a sieve, and any larvae removed with a pipette with aid of flash light, stored in vials and transported to the laboratory.

A tea strainer was used for capturing larvae from containers that were large to drain or manipulate, such as tires or small fixed containers (Manrique-Saide et al. 2011). Aquatic nets were used to collect larvae from large containers such as roof water tanks with low densities of larvae. The net was immersed carefully 7.5 cm beneath the water surface of the container and moved around the perimeter in a downwards spiral, creating a vortex which concentrated the larvae at the bottom center of the container. The larvae were then scooped up in the net, collected and then stored (Manrique-Saide et al. 2011). Immatures were reared to adults and were identified to species under a microscope using identification keys (Gerberg1970, Rueda 2004).

**Data Analysis**

The house index (HI), container index (CI), and Breteau index (BI) were calculated (Wld. Hlth. Org. 2003 follows:

House Index (HI) = (Number of houses infested / Total number of houses inspected) × 100

Container Index (CI) = (Number of positive containers infested/ Total number of containers inspected) × 100

Breteau Index (BI) = (Number of positive containers / Total number of houses inspected) × 100.

A HI > 5% and/or a BI > 20 for any locality is an indication that the locality is Dengue sensitive and therefore adequate preventive measures should be taken. Depending on potential for outbreak, an area can be placed into one of the following four categories based on dengue infection and entomological indices (Sekhon and Minhas 2014)

Priority I: Death due to Dengue confirmed.

Priority II: HI > 5, BI > 20,

Priority III: HI < 5, BI < 20, and

Priority IV: Despite active search, no breeding sites found positive

[Explain how you constructed the map in Figure 1]

Map showing the infestation of *Ae. aegypti* was generated by using Punjab information technology board website. It is a government owned website which also tracks dengue surveillance activities throughout Punjab Province. The positive spots after confirmation were uploaded to the website by using Android App with name “Punjab Anti Dengue” which is linked with that website. The generated map show level of infestation of Rawalpindi city with different colored circles. The description of these circles are given in Fig 1.

The container preference of *Ae. aegypti* was assessed by calculation of the breeding preference ratio (BPR) for each of the 14 types of habita (Kumar et al. 2002), where

**X% =** Total no. of one type of container examined x 100 / Total containers examined

**Y% =** Total no. of one type of positive container x 100 / Total positive containers examined

**BPR (Y%/X%) =** % of positive containers (Y%) / % of examined container (X%)

**Results:**

In the present study, a total of 1,050,298 houses and 10,008,037 wet containers were examined, of which 14,225 houses and 14,985 containers were found positive for *Ae. aegypti* larvae. The highest percentage of positive houses (2.75%), positive containers (0.30%) and Breteau Index (2.92%) were recorded during the monsoon season, followed by the post-monsoon (Table 1).

The red circles in (Fig 1) were areas with highest infestation and it show >100 positive sites in that particular area of the city. It displayed that the areas like Dhoke syedan, 6th road, stadium road and Airport road were with highest positive sites. The yellow circles show area’s with <100 positive and blue circles show <10 positive sites in that particular area.

[describe Fig 1 here and what these points mean for dengue risk as described above. Which areas of the city had the highest infestation].

Although variable among seasons, the highest BPR was recorded for water tanks and room air coolers during the monsoon season, followed by urban trash and discarded tires (Table 2).

**Discussion**

From the present entomological survey, it can be concluded that *Ae. aegypti* is well-establishedin Dhoke syedan, 6th road, stadium road are Airport road area’s of Rawalpindi as shown in Fig 1. [add something about Fig 1 here] Most of areas had a high frequency of larva-positive water tanks and room air coolers which are kept indoors, and are rarely emptied and cleaned, thus favoring the breeding of *Ae. aegypti* mosquitoes. Moreover, urban trash is neither disposed properly nor removedfrequently. This urban trash contains disposable bottles, cups, and plastic bags which serve as small water pockets after every rain and provide *Ae. aegypti* larval habitats near densely populated areas. In addition, used vehicle tires are not disposed of properly and are accumulated at residents providing ideal *Ae. aegypti* larval habitat that effectively hold rain water and rapidly warm in sunlight even during winter.

The water supply in Rawalpindi is irregular and insufficient due to electric power shortages and supply water source limitations. As a consequence, almost every household stores water for one to two days use in containers such as metal drums and plastic buckets that are never completely emptied. This situation favors the breeding of *Ae. aegypti*, especially in the pre-monsoon and post-monsoon seasons when fewer rain water filled containers are available. The best conditions for *Ae. aegypti* mosquito breeding and the highest numbers of positive containers and mosquito larvae were found during the monsoon seasons, followed by the post-monsoon season. At these times most of the potential habitats/containers were filled with fresh water creating breeding sites for *Ae. aegypti*.

Similar results were found in a study conducted in North India were Sekhon and **Minhas** (2014) observed maximum breeding of *Ae. aegytpi* in discarded tires (90.32%) and urban trash i.e. discarded plastic bottles (74.34%) (Wilson and Sevarkodyone 2014). In another study Singh et al. (2008) assessed preferred container of *Ae. aegypti* for breeding and determined among all the habitats the maximum breeding of *Aedes* larvae was identified in coconut shells and discarded tires, followed by junk materials, metal and plastic containers, and earthen pots . Wilson and Sevarkodiyone (2014) studied breeding preference ratio of dengue and chikungunya vectors in Virudhunagar District, Tamil Nadu, South India. BPR was observed to be highest in unused stone grinders (0.56) followed by cement tanks/tubs (0.45) and plastic overhead tank (0.36) (Singh 2011). Getachew et al. (2015) studied breeding sites of *Ae. aegypti* in Dire Dawa, East Ethiopia and the common breeding habitats observed in the study area were tires, barrels, plastic drums, and jerricans.

Because of the relatively high values of the three *Ae. aegypti* larval indices in the present study, Rawalpindi falls in priority III (Sekhon and Minhas 2014). Particularly the areas marked red in the fig 1 showed comparatively high level of infestation and needs utmost attention regarding mosquito control interventions to reduce the chance of disease transmission. [how does the data in Fig. 1 influence intervention or transmission risk]. As in the recent past, there have been confirmed cases of dengue in city and particularly form these areas. The results of our study highlight the need for an intensification of vector surveillance activities in Rawalpindi along with source reduction and public awareness programs that should specifically be directed at the proper management of mosquito breeding containers with special attention to water tanks, air room coolers, discarded tires and urban trash.

**Ethical Considerations**

The study was conducted with approval and coordination of Executive District officer (EDO) Health, Rawalpindi. Names of households were not disclosed to anyone, at any stage of the study or under any circumstances and were used for research purposes only.

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**Table 1: Seasonal distribution and entomological indices of *Ae.aegypti* in Rawalpindi [define HI, CI, BI here or as footnotes.]**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Season** | **Houses Searched** | **Houses Positive** | **Containers Searched** | **Containers Positive** | **1HI %** | **2CI %** | **3BI %** |
| **Pre-Monsoon** | 575692 | 2591 | 5570418 | 2703 | 0.45 | 0.04 | 0.46 |
| **Monsoon** | 302375 | 8334 | 2930508 | 8843 | 2.75 | 0.30 | 2.92 |
| **Post-Monsoon** | 172231 | 3300 | 1507111 | 3439 | 1.91 | 0.22 | 1.99 |
| **Total/Average** | 1050298 | 14225 | 10008037 | 14985 | 1.35 | 0.14 | 1.42 |

1House index (HI): the percntage of houses infested with larvae or pupae.

2Container index (CI): the percentage of water-holding containers infested with larvae or pupae.

3Breteau index (BI): percentage of positive containers in inspected houses.

**Table 2: Breeding preference ratio (BPR) of *Ae. aegypti* in different containerhabitats in Rawalpindi.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of container** | | Water tanks | Room air cooler | Urban trash | Discarded Tire | Animal water trough | Birds water pots | Fridge trays | Construction debris | Metallic drums | Flower pots | AC water collection bottle | Gutter cover hole | Drinking water container | Water pocket of leaked water taps |
| **No. of containers searched** | Pre-monsoon | 798325 | 99780 | 117426 | 27497 | 33309 | 101962 | 569123 | 49627 | 987274 | 1279301 | 58736 | 214104 | 1170319 | 63635 |
| Monsoon | 98536 | 422942 | 14098 | 54891 | 59479 | 17908 | 310430 | 654937 | 101944 | 19687 | 22414 | 44360 | 606625 | 502257 |
| Post-monsoon | 252608 | 30614 | 29353 | 6531 | 5029 | 180563 | 24799 | 273950 | 309464 | 8768 | 48662 | 318886 | 5029 | 9982 |
| **1X%** | Pre-monsson | 14.33 | 1.791 | 2.10 | 0.49 | 0.59 | 1.83 | 10.21 | 0.89 | 17.72 | 22.96 | 1.054 | 3.84 | 21.09 | 1.142 |
| Monsoon | 3.36 | 14.43 | 0.48 | 1.87 | 2.02 | 0.61 | 10.59 | 22.34 | 3.47 | 0.67 | 0.76 | 1.513 | 20.0 | 17.13 |
| Post-monsoon | 16.79 | 2.03 | 1.95 | 0.43 | 0.33 | 12.03 | 1.64 | 18.21 | 20.57 | 0.58 | 3.23 | 21.21 | 0.33 | 0.66 |
| **No. of positive containers** | Pre-monsoon | 2072 | 197 | 47 | 9 | 8 | 23 | 114 | 7 | 125 | 67 | 3 | 8 | 22 | 1 |
| Monsoon | 2849 | 4570 | 103 | 122 | 122 | 19 | 323 | 286 | 43 | 8 | 5 | 6 | 70 | 317 |
| Post-monsoon | 2624 | 277 | 53 | 8 | 6 | 128 | 16 | 167 | 102 | 2 | 102 | 38 | 6 | 1 |
| **2Y%** | Pre-monsoon | 76.65 | 7.28 | 1.738 | 0.33 | 0.29 | 0.85 | 4.21 | 0.25 | 4.62 | 2.47 | 0.11 | 0.29 | 0.81 | 0.03 |
| Monsoon | 32.21 | 51.67 | 1.16 | 1.37 | 1.37 | 0.21 | 3.65 | 3.23 | 0.48 | 0.09 | 0.05 | 0.06 | 0.79 | 3.58 |
| Post-monsoon | 76.30 | 8.05 | 1.54 | 0.23 | 0.17 | 3.72 | 0.46 | 4.85 | 2.96 | 0.05 | 0.31 | 1.104 | 0.17 | 0.02 |
| **3BPR (Y/X)** | Pre-monsoon | 5.34 | 4.06 | 0.82 | 0.67 | 0.49 | 0.46 | 0.41 | 0.28 | 0.26 | 0.10 | 0.10 | 0.07 | 0.03 | 0.03 |
| Monsoon | 9.58 | 3.58 | 2.41 | 0.73 | 0.67 | 0.35 | 0.34 | 0.14 | 0.13 | 0.13 | 0.07 | 0.04 | 0.03 | 0.03 |
| Post-monsoon | 4.54 | 3.95 | 0.78 | 0.53 | 0.52 | 0.31 | 0.28 | 0.26 | 0.14 | 0.09 | 0.09 | 0.05 | 0.52 | 0.04 |

**1X% =** *Total no. of one type of container examined* x 100

*Total containers examined*

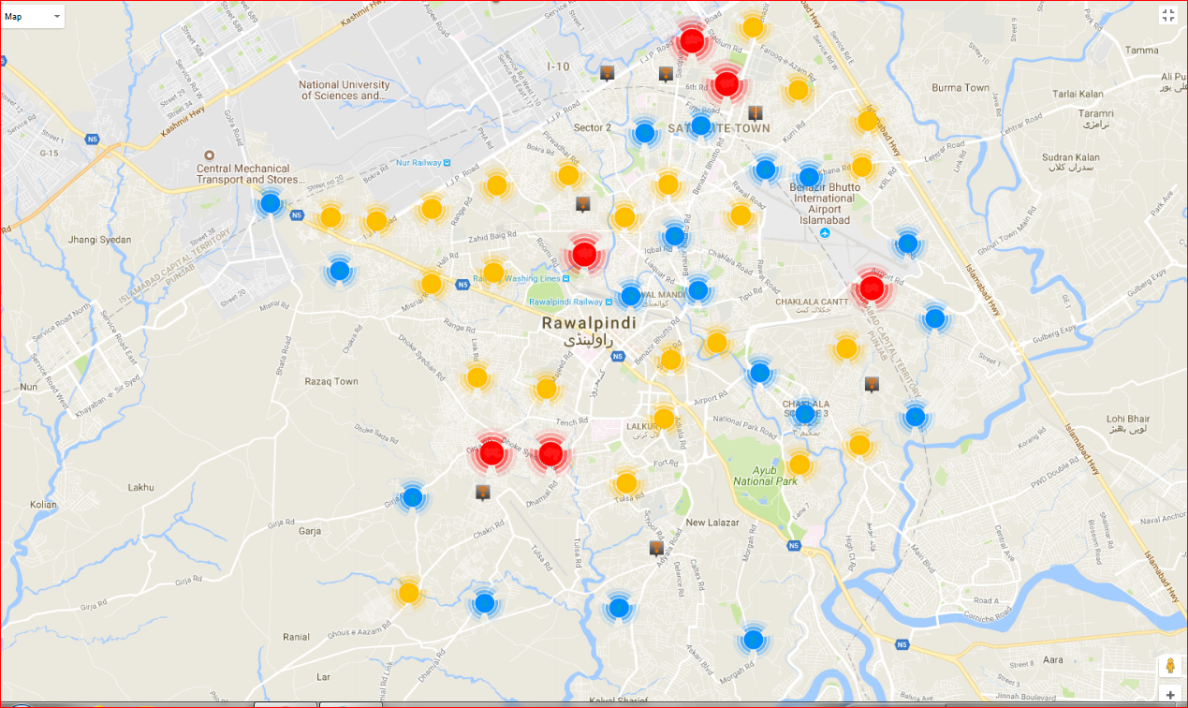
**2Y% =** *Total no. of one type of positive container* x 100

*Total positive container examined*

**3BPR (Y%/X%) =** *% no. of positive containers (Y%)*

*% no. of examined container (X%)*

**Figure 1: Map showing different areasof Rawalpindi found positive for *Aedes aegypti*.**



= area with more hundred aegypti positive spots

= area with less than hundred aegypti positive spots

= area with less than ten aegypti positive spots