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Breeding preference of dengue vectors in Pakistan: An entomological investigation

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Abstract
Dengue fever (DF) and dengue hemorrhagic fever (DHF) are considered important reemerging arboviral diseases in more than 100 tropical and subtropical countries of the world. The Entomological surveillance of Dengue vector was carried out in six highly populated urban areas of Rawalpindi as per the WHO procedure. A total of 492 houses were searched for *Aedes* breeding and 262 houses found positive. About 731 water containers were searched, out of which 281 found positive for *Aedes*. Breeding preference ratio (BPR) was also calculated and discussed. The overall house index (HI), container index (CI), breteau index (BI) were 53.25, 38.44, and 57.11 respectively. Breeding Preference Ratio (BPR) was observed to be highest in water coolers (2.16), Discarded tyres (1.71), underground cement tank (1.69) and air condition water outlet containers (1.56). The results revealed that all the study areas of Rawalpindi city were found to positive for *Ae. aegypti* and *Ae. albobictus*.

Keywords: Dengue, surveillance, Pakistan, breeding preference ratio

1. Introduction
Dengue fever (DF) and dengue hemorrhagic fever (DHF) are considered important reemerging arboviral diseases in more than 100 tropical and subtropical countries of the world [1]. The disease epidemiology is complex in nature and requires understanding of a variety of factors that include weather and environmental changes, [2, 3, 4] vector species composition and behavior, [5, 6, 7] population dynamics and degree of immunity in local population [8, 9, 10].
Eastern Mediterranean Region (EMR) dengue Outbreaks have been documented since 1799 from Egypt [4].
In Pakistan, dengue is emerging as one of the major public health problems, particularly since 2005, threatening the lives of millions of people due to prevailing peculiar socioeconomic conditions and epidemiological situation. Historically, dengue has been endemic in the southern parts of the country. In Pakistan, dengue was recognized for the first time in 1994 in Karachi and one patient out of 145 cases died [11].
Aedes aegypti and *Aedes albopictus* have been considered as the major vectors of dengue in South-East Asia, including Pakistan [12-16]. Both species have been closely associated with human dwellings due to their breeding preference for clean-water domestic habitats [17, 18].
Entomological surveillance, particularly based on larval surveys, provides vital information for better dengue disease management. However, in Pakistan, at present there is no systematic entomological surveillance system, particularly after the 1980s to update the knowledge of vector species and their bionomics.
Entomological surveillance for the dengue vector is used for operational (and research) purposes to determine changes in (1) geographical distribution of vectors (2) monitoring and evaluating control programmes (3) obtaining relative measurements of the vector population over time (4) facilitating appropriate and timely decisions regarding interventions. Vector surveillance may serve to identify areas of high-density infestation or periods of mosquito population increase. In areas where the vector is no longer present, entomological surveillance is critical in order to detect new introductions rapidly before they become widespread and difficult to eliminate.
The new knowledge generated through these investigations will provide a technical basis to design evidence based, community-friendly and sustainable preventive and control measures

against dengue in Pakistan. Hence it is essential to make an inventory of the preferred breeding places of mosquitoes in different places periodically to prevent the outbreak of mosquito borne diseases.

2. Materials and Methods

The Entomological surveillance of Dengue vector was carried out in six highly populated urban areas of Rawalpindi including Dhoke Ratta (33.613088N, 73.046018E), Darya Abad (33.603978N, 73.058258 E), Satellite Town (33.634957N, 73.072018E), Westridge III (33.614300N, 73.022539E), Kamalabad (33.572299N, 73.033350E) and Tench Bhatta (33.581962N 73.034925E) (Fig 1).

The houses were selected by using the simple random sampling. To locate the breeding sites of Aedes female mosquitoes and their larvae various storage containers were searched with the help of dippers and search lights. Different water containers including plastic drums, water coolers, earthen pots, Animal and birds drinking pots, fridge water trays, overhead water tanks, discarded motor tyres and underground cement tanks etc were investigated. A record for breeding habitats and their actual location was maintained. Collected data was analyzed and different indices like, container index (CI), house index (HI), and Breteau index (BI) were calculated as per World health Organization procedure.

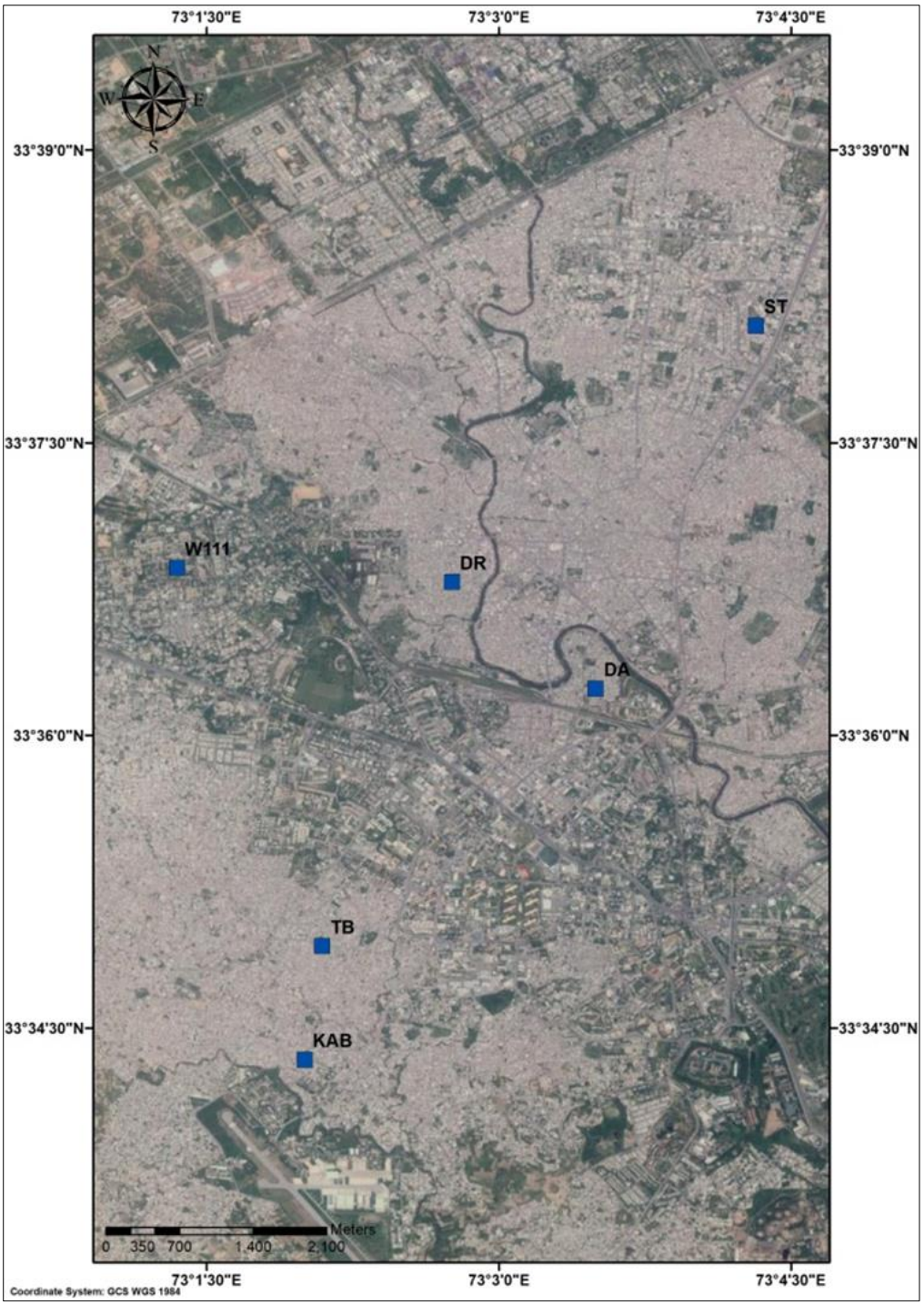

Fig 1: Map showing surveillance sites of six highly populated urban areas of Rawalpindi city including the Dhoke Ratta (DR), Darya Abad (DA), Satellite Town (ST), Westridge III (W111), Kamalabad (KAB) and Tench Bhatta (TB).

Container Index (CI): It is the percentage of water holding containers infested with the larvae or pupae. CI provides information on intensity of breeding.

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

House Index (HI): It is the percentage of houses infested with larvae and/or pupae. HI provides information on extent of breeding. It is calculated as stated hereunder.

$$HI = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \times 100$$

Breteau Index (BI): It is the percentage of number of positive containers per 100 houses inspected. BI is the yardstick for evaluation of control strategy.

$$BI = \frac{\text{Number of positive containers}}{\text{Number of houses inspected}} \times 100$$

The breeding preference ratio (BPR) [9] was calculated to find the container preference for breeding by female *Aedes aegypti*. Adult *Aedes* mosquitoes were collected with the help of aspirators and flash-lights during morning hours (07:00–09:00 hrs.) from tyres, cement tanks, iron pipes, etc. and identified up to species level with the help of standard identification keys [19].

3. Results & Discussion

A total of 492 houses were searched for *Aedes* breeding and found positive in 262 houses. About 731 water containers were searched, out of which 281 found positive for *Aedes*. The results revealed that all the study areas of Rawalpindi city were found to positive for *Ae. aegypti* and *Ae. albobictus*. Breeding habitats of these vectors were observed in all kinds of temporary and permanent water bodies located both indoor and outdoor residential areas. The common breeding habitats observed in the study area were Earthen pots, Water coolers, water plastic containers, plastic drums, Birds drinking pots, Animal drinking pots, Air Conditions water out let containers, fridge water trays, Discarded tyres, overhead plastic tanks, overhead cement tanks and underground cement tanks. Among all the habitats, the maximum positivity for the frequent occurrence of *Aedes* larvae was recorded in water coolers followed by discarded tyres and underground cement tanks. The overall house index (HI), container index (CI), breteau index (BI) were 53.25, 38.44, and 57.11 respectively (Table 2). Breeding Preference Ratio (BPR) was observed to be highest in water coolers (2.16), Discarded tyres (1.71), underground cement tank (1.69) and air condition water outlet containers (1.56) (Table 1).

The presence of mosquito larvae in different habitats shows their ability to survive in a particular habitat and female oviposition preference. Changes in the physio-chemical and biotic characteristics of the habitat may create conditions either favorable or unfavorable for their breeding success, depending upon the range of tolerance of different species. [20, 21] The focus of the present study is to explain the breeding preference of *Ae. aegypti* to different water-holding containers in different populated areas of Rawalpindi.

Among all the habitats, the maximum positivity for the frequent occurrence of *Aedes* larvae was recorded in water coolers followed by discarded tyres and underground cement tanks. *Ae. aegypti* are believed to be associated with man-made artificial habitats in shaded places in human dwellings. [14, 17, 18] Our findings confirm these associations as 100% positive habitats were man-made and domestic in nature. Normally, a underground cemented water tank is 8'x10' in size having 8–12 feet depth and is in common use in these areas, and we observed that these tanks were never emptied completely and mostly not cleaned ever since their construction. Most people make a small hole in their metallic lid to insert a pipe for filling and taking out water, which makes an easy access of *Aedes* mosquitoes to the water for breeding.

Table 1: Breeding preference ratio of dengue vector in urban areas Rawalpindi, Pakistan.

Types of breeding habitats	Examined (X)	X %	With Aedes Larvae (Y)	Y%	Breeding preference ratio BPR (Y/X)
Earthen pots	150	20.52	53	18.86	0.92
Water coolers	35	4.79	29	10.32	2.16
water Plastic Containers	89	12.18	42	14.95	1.23
Plastic drums	91	12.45	15	5.34	0.43
Birds drinking pots	7	0.96	1	0.36	0.37
Animal drinking pots	27	3.69	6	2.14	0.58
Air condition water outlet container	15	2.05	9	3.20	1.56
fridge water trays	43	5.88	2	0.71	0.12
Discarded tyres	73	9.99	48	17.08	1.71
Over Head Plastic tanks	126	17.24	31	11.03	0.64
Over Head cement tanks	12	1.64	4	1.42	0.87
Underground Cement tanks	63	8.62	41	14.59	1.69
Total	731		281		

X- Number of searched breeding places of *Aedes*; X%-Percentage of Breeding places of *Aedes*; Y- Number of larvae in the each breeding containers; Y%-Percentage of *Aedes* in the each container

Table 2: Larval indices and distribution of *Aedes aegypti* breeding habitats at different locations Rawalpindi.

Location	Total Houses	Positive houses	Total Containers	Positive containers	HI (%)	CI(%)	BI (%)
Dhoke Ratta	159	77	225	108	48.43	48.00	67.92
Darya Abad	112	52	115	31	46.43	26.96	27.68
Satellite Town	47	11	77	19	23.40	24.68	40.43
Westridge III	78	46	120	27	58.97	22.50	34.62

Kamalabad	34	29	57	14	85.29	24.56	41.18
Tench Bhatta	62	47	137	82	75.81	59.85	132.26
	492	262	731	281	53.25	38.44	57.11

HI-House Index, CI-Container Index, BI-Breteau Index

4. Conclusion

Since there is a rising incidence of dengue/DHF in Pakistan, particularly since 2005, there is an urgent need: (i) to establish a separate "Dengue Control Cell" within the Ministry of Health as part of overall health system strengthening; (ii) for a mass health information and promotion campaign for the sensitization of local communities for better acceptance of intervention(s), particularly the use of personal protective measures and also to change their behavior for employing improved water-storage practices like proper covering of water-holding containers, use of larvicides, symptoms recognition for prompt treatment seeking, etc.; (iii) for establishing a functional intersectoral mechanism of coordination between all stakeholders for implementation of an integrated vector management approach; (iv) for sensitization of local authorities for regular water supply and proper solid waste management; and (v) for regular capacity building programmes. Operational research on insecticide resistance in dengue vector(s), characteristics of virus, vector(s) densities and bionomics between high- and low-affected areas, rural and urban areas, frequency of host vector contact and disease epidemiology is also strongly recommended, which ultimately would lead to the development of an evidence-based, community-friendly and sustainable disease management strategy in the country.

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