ORIGINAL ARTICLE

Seasonal and spatial quantitative changes in *Aedes aegypti* under distinctly different ecological areas of Lahore, Pakistan

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Abstract

Objective: To find out the variations in larval and adult density of *Aedes aegypti* in different seasons under different ecological conditions.

Methods: This study was undertaken in all the nine towns and the cantonment board of Lahore, Pakistan, during four seasons in 2011, 2012 and 2013. Ovitraps were placed in houses in residential areas and were visited weekly in rainy and post-rainy seasons each year to determine the presence of immature and mature forms of *Ae.aegypti*. Densities of these were measured by ovitrap index and per man hour density, respectively. Correlation coefficient and coefficient of determination between ovitrap index, per man hour density and climatic variables were established. SPSS 19 was used for data analysis.

Results: Ovitrap index and per man hour density values were lower in early rainy season compared to late rainy and early post-rainy seasons. These became lowest in late post rainy season. Strong correlation coefficient and its determination between ovitrap, per man hour density and climatic variables were observed.

Conclusion: Density of immature and mature forms of Ae.aegypti was influenced by environmental degradation.

Keywords: *Aedes aegypti*, Ovitrap, Environmental degradation, Seasonal variation, Ovitrap index (OI), Per man hour density (PMHD). (JPMA 67: 1797; 2017)

Introduction

Dengue is a viral infectious disease prevalent in tropical and sub-tropical regions of the world. It is transmitted by *Aedes* (Ae.) mosquitoes, *Ae. aegypti*.[1] Since its introduction in Pakistan through the port city of Karachi at the start of this century, dengue infection has spread to other parts of the country owing to climate change, rapid urbanisation and environmental degradation.[2] In Lahore, dengue has become endemic since 2006, rising to epidemic levels in 2011 and resulting in enormous public health crisis. The factors responsible for dengue endemicity include lack of proper sanitation, collection of stagnant water in empty small and large containers and puddles, and unattended piles of accumulated garbage/litter and the presence of vegetation in unattended plots near residential areas. Such favourable ecological conditions conducive to mosquito propagation often lead to increased mosquito populations/density during rainy- and post-rainy seasons.[3]

Many studies, based on the extent of urbanisation and vegetation coverage in urban areas have demonstrated the breeding pattern of *Aedes* mosquitoes. Honório et al. in 2006 reported that *Ae. aegypti* and *Ae. albopictus* tend to breed in densely populated urban areas with interspecies competition as well.[4] Both mosquito species remain abundant in or near residences than in deep forests. It has been reported that *Ae. aegypti* is more frequent in highly urbanised and densely populated neighbourhoods, whereas *Ae. albopictus* is more prevalent in rural, suburban and forested urban areas.[5,6] In addition, areas located close to highways with intense traffic can facilitate the introduction and circulation of dengue viruses. In these areas, points, such as gasoline stations, tyre shops and scrap metal yards are strategically important for the surveillance of dengue vectors.[7] The favourable environment for the proliferation of *Ae. aegypti* include water-filled containers, nectar and human blood for egg development, and shady habitats for resting and oviposition.[8-10] These favourable ecological conditions have led to increased mosquito density during rainy and post-rainy seasons.[3]

Many researchers have reported dengue outbreaks in relation to seasonal patterns. Relationship between density of both immature and mature forms of *Ae. aegypti* and climatic variables like temperature, humidity and rainfall have been studied by different researchers. Studies have reported a strong relationship between dengue vector and these variables.[11-13] However, Foo et

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al. pointed out that whenever there is heavy rainfall, there is a possibility that it may flush out eggs and larvae from ovitraps.[11]

In this study, an alternate method like ovitrapindex (OI) method was used instead of traditional methods involving container index (CI), house index (HI) and Breteau index (BI). OI method was economical, reliable, can detect low infestation and assess the affectivity of control measures.[14] Historically, this method was used in the mid-1960s.[15,16] The Pan American Health Organisation (PAHO) and Morato et al. 2005 have advocated the use of ovitraps for monitoring of vector density, presence/absence of immature vectors and to know the efficacy of insecticides.[9,17] So, the results of ovitraps can be used in surveillance and control of *Ae. aegypti* mosquitoes. *Ae. Aegypti* population structure differs in different geographical areas of the world due to different ecological conditions and there is a lack of information regarding oviposition activities of *Aedes* mosquito in Lahore.

The current study was planned to determine the density of immature and mature forms of *Ae. aegypti* in residential areas of the metropolitan city. This study will help to develop guidelines for local control actions to be implemented against this vector. The study also tried to bridge information gap regarding *Ae. aegypti* mosquitoes breeding pattern in relation to seasonal and spatial differences in an urban town of a metropolitan city having potential of dengue outbreak at epidemic levels.

Materials and Methods

This study was undertaken in all the nine towns and the cantonment board (CB) of Lahore, Pakistan, during four seasons (early rainy from 27th to 32nd week, late rainy from 33rd to 38th week, early post-rainy from 39th to 45th week, and late post rainy from 46th to 52nd week) in 2011, 2012 and 2013. The study area and its environmental conditions were assessed in a pilot study before the actual study. Meteorological data including temperature, humidity and rainfall was obtained from the local meteorological office.

Lahore is divided into 10 administrative zones: 9 towns and one CB. Each town is subdivided into union councils (UCs). There are 146 UCs in Lahore and the number of UCs in each town varied from 12-18. From each of the nine towns, 5 UCs and five sites in CB were selected randomly for the survey. Ten houses were selected randomly in each of these UCs for ovitrap placement. The selection of houses involved marking any house as a starting point (labelled as House No. 1) and walking for 50 metres in the direction of a pointed end of pencil, thrown in the air to select the second house and this was labelled as House No. 2. This process continued till the required number of ten houses was achieved. In case of a house not being available (locked or some other reasons), the adjacent house was selected. One ovitrap was placed in each house.

Mosquito larval density was estimated by employing ovitraps, a pot, preferably black in colour that could hold approximately 250ml of water. Female gravid mosquitoes lay eggs on the piece of a white strip (10 x 2.5 cm), placed on the side of pot. These ovitraps were visited weekly to determine the presence of larvae of *Aedes* mosquito. Water having larvae was shifted to the entomology laboratory of the Institute of Public Health (IPH), Lahore, for identification using a key, 'A Catalogue of the Mosquitoes of the World (Diptera, Culicidae).[18] Water devoid of any larvae in ovitrap was thrown. Ovitrap container was washed thoroughly, filled again with fresh water and placed for examination for the next week. Per catalogue, OI was calculated as the percentage positive container/100 households inspected by the following formula:

OI = No. of positive ovitraps/Total No. of ovitraps inspected X 100

Estimation of adult density:

Adult dengue vector mosquito density was determined by per man hour density (PMHD). For this purpose, adult mosquitoes were collected from inside sampled houses as well as from 200-metre radius of the houses where ovitraps were placed. Each collection was made for 15-20 minutes using a battery-operated aspirator and the collected data were expressed as PMHD (World Health Organisation (WHO), 2003) using the expression given below:[19]

$$PMHD = N \times 60/T \times P$$

Where N is the number of adult mosquitoes collected, T is time used up in minutes, and P is the number of people involved in mosquito collection.

Environmental/ecological conditions such as human population density, presence of open drains, presence and extent of vegetation cover and existence of open vacant lands (commonly used for garbage and litter disposal) around each ovitrap placement site were recorded during the study period. Average air temperature, relative humidity and total rainfall during the studied seasons were collected from local meteorological office.

Data collected was screened and presented in tabulated form and analysed using SPSS 19. Correlation of entomological indices (OI and PMHD) with each other and with climatic and environmental variables was determined by finding correlation coefficient (r) and coefficient of determination (r2) values. P<0.5 was considered as statistically significant.

Formal permission to conduct this study was obtained from the ethics committee of the IPH, Lahore. Verbal consent from occupants of the sampled houses was obtained prior to indoor placement of ovitraps and for capturing of adult mosquitoes. Confidentiality of collected personal information was maintained and personal identity of the households was decoded for security.

Results

A total of 604 ovitraps were placed. OI was highest in late rainy and early post-rainy seasons while it was lowest in early rainy seasons in all years. Town-wise distribution of OI showed that in 2011, the highest values (15.66 and 12.85) were recorded in Aziz Bhatti Town in both late rainy and early post-rainy seasons, respectively. In 2012, OI value was highest (2.6) in the same town in late rainy season while high value (0.85) was recorded in four towns (Aziz Bhatti, Iqbal, Nishter and Wagha) in early post-rainy season. In 2013, the index was highest (3.66 and 7.4) in Aziz Bhatti Town and Data Ganj Bakhsh Town in late rainy season and early post-rainy seasons, respectively (Table-1).

Table-1: Ovitrap Index (OI) in all towns of the Lahore from 2011-2013.

Year / Season	Town									
	Aziz Bhatti	Data Ganj Bakhsh	Ravi	Shalamar	Gulberg	Samanabad	Iqbal	Nishter	Wagha	Cantonment Board
2011										
Early rainy season (Wk: 27-32)	3.33	1	0.3	1.66	0.33	1.33	0	1	1	1.33
Late rainy season (Wk: 33-38)	15.66	11	11.3	12	10.66	11.33	9.33	10.33	11	10
Early post rainy season (Wk: 39-45)	12.85	13.4	4	5.71	3.71	4.85	8.28	5.71	5.71	6.85
Late post rainy season (Wk 46-52)	2	1.42	1.4	0	0	0	0.28	0.57	0.28	0.57
2012										
Early rainy season (Wk: 27-32)	0	0	0.3	0	0	0	0	0	0	0.66
Late rainy season (Wk: 33-38)	2.6	2	1.3	1	1.33	1	0	1.66	0.66	1
Early post rainy season (Wk: 39-45)	0.85	2	0.8	0	0	0.28	0.85	0.85	0.85	0.57
Late post rainy season (Wk 46-52)	0	0	0	0	0	0	0	0	0	0.28
2013										
Early rainy season (Wk: 27-32)	0	0	0	0	0	0	0	0	0.33	1
Late rainy season (Wk: 33-38)	3.66	1.6	0	1.66	0.66	0.66	0	1.33	2.33	5.66
Early post rainy season (Wk: 39-45)	3.14	7.4	1.4	6.57	4.57	3.42	3,71	6.57	1.42	3.14
Late post rainy season (Wk 46-52)	4	6.2	1.4	0.85	0.57	0	1.42	0.85	0	0

Table-2: Per Man Hour Density (PMHD) in all towns of the Lahore from 2011-2013.

Year / Season	Town									
	Aziz Bhatti	Data Ganj Bakhsh	Ravi	Shalamar	Gulberg	Samanabad	Iqbal	Nishter	Wagha	Cantonment Board
2011										
Early rainy season (Wk: 27-32)	79.6	34.4	2	34.8	24.4	30	34	40.8	33.2	32.4
Late rainy season (Wk: 33-38)	166.4	177.2	108.8	138.4	232.4	154	140.4	176.4	146.8	148
Early post rainy season (Wk: 39-45)	140.1	195.4	101.7	122.4	106.22	101.14	150.8	153.94	128.45	105.94
Late post rainy season (Wk 46-52)	32.4	55.8	53.4	18.8	7.2	10.97	20.11	23.65	9.6	25.1
2012										
Early rainy season (Wk: 27-32)	35.6	0	24	35.6	26	32	36	26.8	31.6	19.2
Late rainy season (Wk: 33-38)	79.6	43.6	84	92.4	98.66	114.06	122	122	133.6	149.2
Early post rainy season (Wk: 39-45)	77.1	33.2	97.2	133	118.62	124.18	126.51	128.91	84	155.54
Late post rainy season (Wk 46-52)	6.8	0	13.3	41.1	7.54	15.77	19.88	6.85	0.68	27.08
2013										
Early rainy season (Wk: 27-32)	41.6	4.4	4.4	12.8	57.6	45.2	76.07	31.2	36.8	54.8
Late rainy season (Wk: 33-38)	90.4	56.8	15.2	64	192	110.48	166.8	133.2	160.4	174
Early post rainy season (Wk: 39-45)	113.1	162.8	30.1	151.5	133.37	184.8	138.51	201.94	173.02	154.28
Late post rainy season (Wk 46-52)	110.4	115.7	20.9	51.3	17.14	28.8	17.82	40.68	28.82	39.77

Wk - Week.

Table-3: Seasonal correlation between various factors associated with occurrence of dengue in Lahore, Pakistan.

Variable	Variable	Season							
		Early rainy		Late rainy		Early post rainy		Late post rainy	
		r	r^2	r	r^2	r	r^2	r	r^2
OI	PMHD	0.497**	0.247	0.552**	0.272	0.534**	0.285	0.822**	0.675
	Temperature	0.454**	0.206	-0.234**	-0.054	0.444**	0.197	0.270**	0.072
	Humidity	0.272**	0.073	-0.008	-0.000	0.064	0.004	-0.095	-0.009
	Rainfall	0.311**	0.096	-0.094	-0.008	0.216**	0.046	-0.110	-0.012
PMHD									
	Temperature	-0.436**	-0.190	-0.219	-0.047	0.617**	0.380	0.469**	0.219
	Humidity	0.208**	0.043	-0.182*	-0.033	0.246**	0.060	-0.167*	-0.027
	Rainfall	0.492**	0.242	-0.004	-0.000	0.380**	0.144	-0.135	-0.018

'r'- correlation coefficient. 'r2' - coefficient of determination.
** 'r' strongest.
OI: Ovitrap index.
PMHD: Per man hour density.

As far as the density of adult mosquitoes measured by PMHD was concerned, the high values were observed in late rainy and early post-rainy seasons in 2011, 2012 and 2013. Town-wise distribution of PMHD in 2011 showed highest values (232.4, 177.2 and 176.4) in Gulberg, Data Ganj Bakhsh and Nishter Town, respectively, in late rainy season and 195.4 in Data Ganj Bakhsh town in early post-rainy season. In 2012, in late rainy season CB and Wagha Town showed highest values (149.2 and 133.6, respectively) of PMHD while CB, Nishter and Iqbal Town had highest (155.54, 128.91 and 126.51, respectively) PMHD in early post-rainy season. In 2013, Gulberg Town and Nishter Town had highest PMHD (192 and 201.94) values in late rainy season and early post-rainy seasons, respectively (Table-2).

There was a strong correlation (r =0.552, 0.534 and 0.822) between OI and PMHD in late rainy, early post-rainy, late post-rainy seasons, respectively. Strong relationship between OI and temperature was also observed in all seasons. Humidity correlated with OI (r =0.272) only in early rainy season while rainfall had significant correlation (r=0.311 and 0.216) in early rainy and early post-rainy seasons. PMHD had negative correlation (-0.436 and -0.219) with temperature in early rainy and late rainy seasons, respectively (Table-3).

Discussion

Results indicated that OI values were lower in early rain season than late rainy and early post-rainy seasons. The reason was that during this period, air temperature, humidity and rainfall did not favour proliferation of mosquitoes in the first place. Secondly, food was not abundant inside houses in comparison to open places during early seasons so gravid mosquitoes did not lay eggs in pots placed inside houses. As the time passed, food quantity in open places reduced and humidity and rainfall increased. Thus, female mosquitoes moved inside and started laying eggs. Due to this, OI started to increase in late season and became highest in early post-rainy seasons in 2011 and 2012. In 2013, this pattern was changed slightly as comparatively high OI values were observed in early post rainy seasons, especially in Data Ganj Bakhsh Town and Nishter Town. One of the main reasons was high rainfall ranging from 223.51-497.29 mm. Comparable results were portrayed by Manzoor et al. and Hakim et al.[20,21]

As far as the adult density of the vector in early rain season measured by PMHD is concerned, its incidence was also not high in early rainy season in all sampling years. This could have been due to longer time taken for adult mosquitoes to develop from eggs, larvae and pupae because in early rainy season the climatic conditions were not conducive for rapid development of the vector to adult stage. In late rainy season, due to reduced availability of food outdoors, adult mosquito population shifted to houses. Thus, PMHD inside houses increased; its value was highest in Data Ganj Bakhsh Town, CB and Nishter Town in 2011, 2012 and 2013, respectively. In late post-rainy season as air temperature (range: 15.8-17.4°C), relative humidity (range: 15.8-17.4°C) and total rainfall (range: 0-75.18 mm) declined significantly compared to earlier seasons, climatic conditions no longer remained favourable for the proliferation of mosquitoes and as a result PMHD values were relatively much lower in this season for all three years.

As reported by Dibo et al., 2008 climatic variations alone do not explain fully *Ae. aegypti* distribution and environmental conditions surrounding the oviposition sites like presence of trash, empty bags and broken earthenware with little collection of water should also be looked for in all selected places.[22] Results of this study showed that although these conditions were present in all towns, they were particularly severe in certain towns. In 2011, these worst conditions were observed in Aziz Bhatti Town in early rainy season, Gulberg and Data Ganj Bakhsh Towns in both late rainy and early post-rainy seasons. In 2012, CB and Shalamar Town were worst as far as the environmental conditions were concerned in late rainy, early post-rainy and late post-rainy seasons. In 2013, these debilitated conditions were present in Aziz Bhatti, Data Ganj Bakhsh, Shalamar, Nishter and CB.

Results portrayed by Braks et al. revealed that the abundance and spatial distribution of *Ae. aegypti* mosquitoes are related to the effects of anthropogenic changes on the environment.[6] These results were not expressed through GPS by the author. Serpa et al. 2013 pointed out in their study that ovitraps were positive three times more in places where environmental conditions were found deteriorated.[23] Padmanabha et al. 2012 also reported a significant seasonal and spatial variation in *Ae. aegypti* abundance.[24]

Increased ovitrap positivity and PMHD due to favourable climatic variable's like moderate temperature, a lot of humidity and high rainfall, demonstrated in the present study, especially in late rainy and early post-rainy seasons was in accordance with the findings of the studies conducted by researchers.[7,8] Mogei et al. 1988 on the other hand, sided on increased rainfall. According to them, increased rainfall along with environmentally deteriorated conditions around human settlement was the main reason for increased vector abundance of both immature and mature forms of *Aedes* mosquitoes.[25]

This study also revealed strong correlation between OI and PMHD in late rainy, early post-rainy, late post-rainy seasons, respectively.

Strength of this study was its extensive coverage as it was conducted in the whole city. Moreover, it was carried out for a duration of three consecutive years from 2011-2013; the year 2011 being the peak period of dengue epidemic in Lahore. Additionally, for estimation of immature population of *Aedes* mosquitoes, an alternate method, i.e. OI, was used instead of the conventional methods as OI was more precise, accurate and reliable. Also, the relationship between climatic factors, *Aedes* mosquito's profusion and distribution identified in this study can be valuable for efficient entomological surveillance and ultimately its effective control. Weakness of the study was non-collection of other varieties of prevalent mosquitoes, as it would have represented a much wider perspective.

Further studies can be based on the information extracted from OI, PMHD and climatic variables (temperature, humidity and rainfall) and can further be correlated with minimum infection rate (MIR) in mosquitoes for assessing the incidence of dengue infection in any community.

Conclusion

Abundance and occurrence frequency of *Ae. aegypti* had a positive correlation with increased level of unplanned urbanisation, commercial activities, unhygienic sanitation, favourable temperature, humidity and rainfall.

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