SEASONAL PREVALENCE OF *AEDES AEGYPTI* IMMATURES IN KOLKATA, INDIA

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Abstract. Dengue and dengue hemorrhagic fever (DHF) is recurring in India in recent years, affecting many cities including Kolkata (Calcutta). In view of this, a survey of three years was carried out on the prevalence of immatures of the vector mosquito *Aedes aegypti* in the city of Kolkata. Mosquito immatures were found throughout the year with fluctuating densities in the habitats surveyed. An index of density of immatures $(I/S)_m$ employed in the study revealed that in the months of August and September a peak in abundance occurs, irrespective of the habitats, with the values ranging between 1.12 and 11.4. The $(I/S)_m$ values were low during the months of April and May ranging between 0.4 and 3.8. As larval habitats, earthen containers and the sewage drains were positive throughout the year, while during the months of December to February, air-coolers and conditioners as habitats remained totally dry, without immature *Ae. aegypti*. The number of positive sites and monthly density between the habitat types were significantly different with an *I/S*- value (for a year) ranging between 2.64 (air-coolers) and 4.97 (earthen containers). The survey, while providing preliminary baseline data on immature abundance in Kolkata, calls for continuous entomological surveillance using standard protocols as carried out in Thailand for the vector management program against dengue.

INTRODUCTION

Apart from other mosquito-borne diseases, such as malaria and Japanese encephalitis (WHO 1997a, 2001), in recent years, the outbreaks of dengue and dengue hemorrhagic fever (DHF) in New Delhi (Sharma *et al,* 2005), Kolkata and other places of India have become a severe public health concern. The mosquito *Aedes aegypti* (Linnaeus 1762) is a vector for dengue and DHF and is common in Kolkata, along with 33 mosquito species (Pramanik and Raut, 2000). Certain vector control programs against this mosquito species have been successful in theory and practice, but the outbreaks of dengue and DHF in India recur. Apart from other factors, the entomological baseline regarding the vector mosquito *Ae. aegypti* forms an important parameter in formulating various control strategies (WHO 1997b, 1999; Focks, 2003). Compared to the National Capital Territory, Delhi (Katyal *et al*, 1996, Ansari and Razdan, 1998; Sharma *et al,* 2005), the city of Kolkata has fragmentary records on the prevalence of *Ae. aegypti* (Pramanik *et al,* 1992; Pramanik and Raut, 2000, 2002) far from the systematic studies carried out in Thailand (Strickman and Kittayapong, 2003). The present study is intended to provide a primary baseline data on immature *Ae. aegypti* in Kolkata as a prelude to surveillance of the entomological factors related to dengue and DHF.

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MATERIALS AND METHODS

Study area

A survey of the larval abundance of *Ae.*

aegypti was carried out in three selected localities (Belghoria, Kasba, and Ballygunge) of Kolkata. Localities were selected irrespective of previous or current dengue and DHF in the city. The survey of these selected regions of the city was carried out between October 2003 and September 2006.

Sampling methods

Four different types of breeding habitats were studied: 1) earthen containers, 2) plastic containers (including polythene bags and objects), 3) sewage drains, and 4) air coolers and conditioners, based on WHO (1999) and Pramanik and Raut (2000, 2004). In each location random sampling of the larval stages was carried out monthly taking at least 20 subjects of each type of the breeding habitat. Sampling of immatures were done using a hand net or scooping the whole contents of the container or by dipping with a plankton net of 0.02 mm mesh size (for sewage drains). The immatures sampled were placed in beakers of appropriate volume or plastic jars of 1 liter volume and brought to the laboratory for counting and recording of larva and pupa of *Ae. aegypti*. Data regarding the number of immatures were used to measure the immature density per month per habitat following Strickman and Kittayapong (2003) with certain modifications using the following formulae:

$I/S_m = TI_m/TS_m$, where I/S_m = immature density (immature per sample; sample = 20/ habitat type), TI_m = total immatures in the sample, TS_m = total sample

$I/S = (\sum TI_m)/(\sum TS_m)$, where I/S = immatures per sample in a year.

$D_m = (I/S_m)/(I/S)$; where D_m = deviation in immature density for a particular month, m

The habitats positive for immatures were also noted equivalent to the container index (WHO, 1999). Data on the water temperature were also noted for each habitat.

Statistical analysis

The data obtained for immatures per habitat type and month were subjected to factorial ANOVA to infer the differences, if any, for the densities of immatures in the containers and during the months of the year. Data recorded on the number of positive sites were subjected to the Friedman non-parametric chi-square test and one sample *t*-test to infer the difference in respect to the habitats. The paired sample *t*-tests were applied to larval densities for the months in the year in the habitats, to justify the differences in density if any. Regression analysis and correlations were carried out on the density of immatures among the habitats as well as with the water temperature. Also, a population level estimate of the correlates was tested from the sample correlation values using the *t-statistic*, $t = r/S_r$, (where r is the coefficient of correlation and S_r is the standard error of r calculated with n - 2 degrees of freedom) and the null hypothesis Ho: p = 0, *ie* no correlation between the variables in the population. This would justify the estimate of density in the sample and the population. The statistical analysis was carried out following Zar (1999).

RESULTS

Larval sampling confirmed the occurrence of *Ae. aegypti* mosquitoes throughout the year in Kolkata, India. The number of immatures collected from different habitats throughout the year varied greatly (Table 1). The index of abundance, expressed in terms of immature density per site per month $(I/S)_m$ is presented in Table 2. The *(I/S)* values for the earthen container habitats ranged between 4.62 and 5.18 with a mean of 4.97±0.18 (SE); for the plastic container habitats it ranged between 2.8 and 3.18 with a mean of 3.04 ± 0.12 (SE); for the sewage drain habitats, it ranged between 4.48 and 4.72 with a mean of 4.58 ± 0.07; and for the air-cooler habitats, it ranged between 2.52 and 2.89 with a mean of 2.64 ± 0.12. The mean number of positive sampling units, (sites that were found positive for *Ae. aegypti*

Table 1
Monthly number (mean ± SE) of *Ae. aegypti* immatures collected from different types of breeding habitats, in Kolkata, India (n = 3 years).

Month	Habitat type			
	Earthen	Plastic	Sewage	Air coolers
January	44.67 ± 3.84	17 ± 1.73	41.33 ± 4.7	0 ± 0
February	102.67 ± 9.02	57 ± 3.51	103 ± 6.66	0 ± 0
March	85 ± 5.86	49.67 ± 3.18	71.33 ± 12.78	37.33 ± 5.46
April	69.67 ± 8.69	8 ± 0.58	59.33 ± 2.33	19.67 ± 3.38
May	49.33 ± 6.89	8.33 ± 1.33	21.33 ± 5.81	7.67 ± 2.19
June	86 ± 3.46	43.33 ± 5.84	70.67 ± 6.12	58.67 ± 6.89
July	105.67 ± 8.11	94 ± 7.09	51.33 ± 0.88	102.67 ± 9.24
August	127.67 ± 6.01	88.67 ± 9.28	22.33 ± 1.45	107.33 ± 9.7
September	152.67 ± 7.42	121 ± 3.46	216.67 ± 3.18	126.33 ± 8.09
October	191 ± 7.94	126.33 ± 3.53	228 ± 9.54	128 ± 3.61
November	109.67 ± 4.98	97 ± 3.51	151.33 ± 3.84	85.67 ± 4.33
December	69.67 ± 3.48	19 ± 1.73	62 ± 7.21	0 ± 0

Table 2
Immature *Ae. aegypti* collected per sample per month $[(I/S)_m \pm D_m]$ in Kolkata, India.

Month	Habitat Type			
	Earthen	Plastic	Sewage	Air coolers
January	2.23 ± 0.45	0.85 ± 0.28	2.07 ± 0.45	0 ± 0
February	5.13 ± 1.03	2.85 ± 0.94	5.15 ± 1.12	0 ± 0
March	4.14 ± 0.83	2.48 ± 0.82	3.73 ± 4.36	1.87 ± 0.67
April	3.48 ± 0.7	0.4 ± 0.13	2.97 ± 0.65	0.98 ± 0.35
May	2.47 ± 0.5	0.42 ± 0.14	1.07 ± 0.23	0.38 ± 0.14
June	4.03 ± 0.86	2.17 ± 0.71	3.53 ± 0.77	2.93 ± 1.04
July	5.28 ± 1.06	4.7 ± 1.55	2.57 ± 0.56	5.13 ± 1.83
August	6.38 ± 1.28	4.43 ± 1.46	1.12 ± 0.25	5.37 ± 1.91
September	7.63 ± 1.54	6.05 ± 1.99	10.83 ± 2.36	6.32 ± 2.25
October	9.55 ± 1.92	6.32 ± 2.08	11.4 ± 2.49	6.4 ± 2.28
November	5.48 ± 1.1	4.85 ± 1.49	7.57 ± 1.65	4.28 ± 1.52
December	3.65 ± 0.73	0.95 ± 0.31	3.1 ± 0.68	0 ± 0

immatures out of 20 surveyed sites per habitat type per month) is shown in Fig 1. The number of positive sites for a particular habitat differed significantly among the months as revealed by the one-sample *t*-tests (for earthen container habitats, t = 10.879, p<0.0001; for the plastic container habitats, t = 7.061, p<0.0001; for the sewage drain habitat, t = 9.633, p<0.0001; and for the air cooler habitats, t = 4.795, p<0.001: all at df = 11). Further, the non-parametric test (Friedman test) revealed that the habitat types were significantly different in terms of the number of positive sites (chi-square χ^2 = 9.974, df = 3, p<0.02). The sewage drains provided a larger space as well as a resource in all the sampled sites, while the air-coolers and air-conditioners were observed to provide the least space

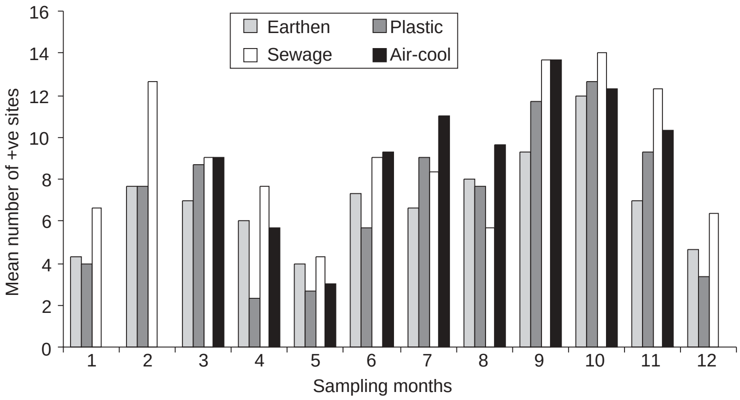

Fig 1–Mean number of positive sites recorded from the sampling of different breeding habitats of *Ae. aegypti* in Kolkata, India.

and resources. During the winter (December-February), the air-coolers and conditioners were completely dry and no immatures were noted. A maximum of 228 immatures in a sampling unit were collected from sewage drains during the month of October. The number of positive sites and densities of *Ae. aegypti* immatures for all the four different habitats were noted to reach a peak during the month of October. During the summer, between March and May, the population was the same found to be low. Though this pattern was the same for all the habitat types, the density of immatures varied between the habitats, possibly due to the habitat sizes and quality. The paired sample *t*-test of the density of immatures in the habitats varied significantly between the earthen and plastic containers and air-coolers, but not between the plastic and air-coolers and between sewage drains and earthen containers (Table 3). The water temperature and density of immatures were positively correlated (for earthen habitats, $r = 0.159$; for plastic container habitats, $r = 0.098$; for sewage drains, $r = 0.080$; for air-coolers, $r = 0.312$; $df = 34$), but statistically insignificant. However, among the habitats the density of immatures collected were positively correlated and the *t*-values were significant ($p<0.001$), confirming similarity in the correlation estimates of the samples and the population (Table 3). This supports the hypothesis that the prevalence of *Ae. aegypti* immatures follows symmetry in monthly fluctuation in the different habitats. The one-way ANOVA revealed that a significant difference ($p<0.0001$) exists among the habitats in respect to the density of immatures (for earthen, $F=41.44$; for plastic, $F=95.19$; for sewage, $F=127.5$; for air-condition/air-coolers: $F=86.17$; for all the $df_{(v1/v2)}=11,35$). The two-way factorial analysis for immature density of *Ae. aegypti* showed significant difference ($p<0.0001$) among the habitat types as well as months and habitat and month interactions (for month, $F=271.69$, $df_{(v1/v2)}=11,96$; for habitats, $F=170.49$, $df_{(v1/v2)}=3,96$; for month* habitat interactions, F-22.73, $df_{(v1/v2)}=33,96$).

DISCUSSION

Based on the results of the survey of immatures of *Ae. aegypti* in Kolkata, it can be concluded that the mosquito is abundant throughout the year with the density varying among the months and among the breeding habitats. The peak in density between the months of August and November coincides with the prevalence of the dengue and DHF in Kolkata. The trend for larval abundance was similar to Delhi (Katyal *et al,* 1996; Ansari and Razdan 1998; Sharma *et al,* 2005), where a peak in larval density was observed in the rainy season (July to September). The density of *Ae. aegypti* immatures was dependent on the type of habitat. The size, resource content and availability of habitats were factors influencing the density of immatures. Sewage drains, followed by earthen containers, had more immatures than air-coolers and plastic con-

Table 3A
Results of paired sample *t*-test and regression equations on the density of immatures in different habitat types.

Pair	*t*-value	df	Regression equation	$F_{(1,35)}$	r^2
1. Earthen and plastic	12.514^{c}	35	y = 44.94 + 0.89x	153.27^{b}	0.82
2. Earthen and sewage	1.04^{NS}	35	y = 56.07 + 0.47x	50.29^{b}	0.6
3. Earthen and air-coolers	9.337^{c}	35	y = 60.28 + 0.7x	79.52^{b}	0.7
4. Plastic and sewage	3.824^{b}	35	y = 20.01 + 0.45x	36.52^{b}	0.52
5. Plastic and air-coolers	1.329^{NS}	35	y = 17.7 + 0.77x	168.62^{b}	0.83
6. Sewage and air-coolers	3.735^{b}	35	y = 47.15 + 0.79x	17.46^{b}	0.34

Table 3B
Results of correlations of the density of immatures in different habitat types, in the sample (r) and significance with the population estimate (t).

Pair	r	df	$t = r/S_r$
1. Earthen and plastic	0.905^{a}	34	12.397^{b}
2. Earthen and sewage	0.772^{a}	34	7.117^{b}
3. Earthen and air-coolers	0.837^{a}	34	8.911^{b}
4. Plastic and sewage	0.72^{a}	34	6.05^{b}
5. Plastic and air-coolers	0.912^{a}	34	12.964^{b}
6. Sewage and air-coolers	0.582^{a}	34	4.173^{b}

[a]Significant at $p<0.01$, [b]Significant at $p<0.001$, [c]Significant at $p<0.0001$

tainers, in the peak season. The immature density per sample per month (I/S_m) values for the habitats supports this. However, the relative importance of these habitats from the control viewpoint is different, considering their location peri domestic or intra-domestic. The air-coolers and air-conditioners and the majority of the plastic containers were within the buildings, while the others were adjacent to human habitations. Thus even if these containers had a lower density of immatures the control measures could not be same as that of the peri-domestic containers. In this city, apart from the plastic and earthen containers, the sewage drains are natural habitats of the predatory mosquitoes *Toxorhynchites splendens* (Pramanik and Raut, 2003, 2004), providing fair chance for biological control of the immatures.

Entomological surveillance in relation to mosquito-borne diseases is primarily aimed at judging the changes in geographical distribution and density of vectors over time and facilitating the requisite decision regarding control (WHO, 1997b). Several indices are known that can be used to detect and monitor immature abundance. Selection of a method depends on the aim of the study (Focks, 2003). However, the biology of vectors in relation to abundance and seasons is an important determinant of the intensity of the disease. Several models have been developed where the data on these parameters in assessing the risk of transmission of the disease are included (Strickman and Kittayapong, 2003). A detailed assessment was made on the potential of the vector in disease transmission with a survey of *Ae. aegypti* immatures in Thailand, incorporating all these parameters (Strickman and Kittayapong, 2002, 2003). In the present study the immature density index was used to reveal the differential level of infestation over the months and the type of habitats. Though this provides a preliminary record of the abundance of immature *Ae. aegypti* in the city of Kolkata, further studies utilizing the demographic and biological components would provide a more appropriate basis for vector management in relation to dengue and DHF.

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