

# A study on container breeding mosquitoes with special reference to *Aedes (Stegomyia) aegypti* and *Aedes albopictus* in Thiruvananthapuram district, India

K. Vijayakumar<sup>1</sup>, T.K. Sudheesh Kumar<sup>1</sup>, Zinia T. Nujum<sup>1</sup>, Farook Umarul<sup>2</sup> & Anu Kuriakose<sup>1</sup>

<sup>1</sup>Department of Community Medicine, Government Medical College, Thiruvananthapuram; <sup>2</sup>Directorate of Health Services, Kerala, India

## ABSTRACT

**Background & objectives:** The district of Thiruvananthapuram reports the maximum number of cases of dengue in the state of Kerala. To determine the larval diversity, density and breeding site preferences of *Aedes* mosquitoes, during pre-monsoon and monsoon periods in urban and rural areas of Thiruvananthapuram district.

**Methods:** Based on the daily reports of dengue cases, 70 clusters were identified in Thiruvananthapuram district. A cross-sectional larval survey was done in the domestic and peri-domestic areas of 1750 houses, using the WHO standard techniques. The larval indices were calculated, and the larvae were identified by using taxonomic keys. Urban and rural differences and the variations during pre-monsoon and monsoon seasons were also studied.

**Results:** In the surveyed houses, 15% had mosquito breeding, with 88% having *Aedes* larvae. The house index, container index and the breteau index were 13.08, 13.28 and 16.57%, respectively. About 86% of the clusters were found positive for *Aedes albopictus* and 11% for *Ae. aegypti*. *Aedes albopictus* was distributed almost equally in rural and urban clusters, whereas the distribution of *Ae. aegypti* was significantly higher in urban areas ( $p = 0.03$ ). The most common water holding containers found (outdoor) were of plastic, followed by coconut shells. The breeding preference ratio was highest for tyres. Significantly lesser positivity was found for containers during monsoon period when compared to pre-monsoon period.

**Conclusion:** The geographical distribution of *Ae. albopictus* is significantly high in peri-domestic areas and, therefore, its epidemiological role in the widespread disease occurrence needs to be studied. The discarded tyres being the most preferred breeding sites, where IEC activities will help in source reduction.

**Key words** *Aedes aegypti*; *Ae. albopictus*; breeding; dengue; entomology; habitat; surveillance

## INTRODUCTION

Dengue is one of the major and fast emerging tropical mosquito borne diseases. It is a public health problem which has spread throughout tropical and sub-tropical regions of the world<sup>1</sup>. Dengue is endemic in South-east Asia, the Pacific, East and West Africa, the Caribbean and the Americas. Dengue, with its two clinical severe manifestations, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) poses a serious health concern in India and is reported from more than 20 states in the country<sup>2–4</sup>. The dengue virus which belongs to the family flaviviridae has four serotypes. They are transmitted by the bite of mosquitoes of the genus *Aedes (Stegomyia)*<sup>5–7</sup>. Even though *Ae. aegypti* Linn. (Diptera: Culicidae) is found to be the principal vector of dengue virus, *Ae. albopictus* is also becoming a competent vector for dengue viruses<sup>8–9</sup>.

Dengue fever was first reported in Kottayam district of Kerala state in 1997. The first epidemic was reported in 2003 with 3546 cases and 68 deaths, representing the

highest number of deaths due to dengue reported in India, and Thiruvananthapuram district was the worst affected. Over the years, the reported cases of dengue have been increasing in Kerala<sup>10</sup>. Kerala is now hyper endemic for dengue with presence of multiple serotypes, high rates of co-infection and local genomic evolution of viral strains<sup>11</sup>. The district of Thiruvananthapuram reports the highest number of cases with 40–50% cases reported from a corporation area of the district<sup>10</sup>. In this context, mosquito larval investigation was carried out in Thiruvananthapuram district, in areas from where dengue cases were reported. This communication presents the results of *Aedes* larval profiling in terms of larval diversity, density (as indices) and breeding source specificity in rural and urban areas during pre-monsoon and monsoon periods.

## MATERIAL & METHODS

The study was carried out in 70 different locations or clusters in the district from which confirmed cases were

reported. Daily reports of confirmed cases of dengue in Thiruvananthapuram district were obtained from the District Medical Officer. Clusters were selected, according to this information. Each cluster comprised of 25 houses around the confirmed case. A door-to-door cross-sectional entomological survey was carried out in houses and peridomestic areas to detect *Aedes* larval breeding with a view to study the level of infestation of areas with *Aedes* larvae and to assess the high risk areas in the district prone to dengue/DHF outbreak<sup>12</sup>. The larval collections were made in each locality, by using dipping and pipetting methods, to find out the *Aedes* breeding in all the water filled containers present in and around the houses and their premises in study areas<sup>13</sup>.

The data on larval collections were recorded in the pre-designed and pre-tested survey forms. The larval identification was done by using the taxonomic key<sup>14</sup>. The data were analyzed and different indices like house index (HI), container index (CI) and breteau index (BI) were calculated. Container preference of *Aedes* larval breeding was also assessed by calculation of breeding preference ratio<sup>12</sup>. The differences in breeding found in urban and rural areas during monsoon and pre-monsoon seasons were tested for significance by using chi-square test.

The consent from health authorities and the necessary ethical approval from the Institutional Ethical Committee of Medical College, Thiruvananthapuram (IEC No. 3/44b/2011/MCT), was obtained before the start of the study.

## RESULTS

A total of 1750 houses (25 houses in 70 clusters) were surveyed. Out of these, 15% (261/1750) houses had mosquito breeding sites in their premises and 88% (229/261) of these houses had *Aedes* breeding. Altogether, 2183 water holding containers were identified, of which 329 (15%) had mosquito breeding. Out of these, 290 (88%) had *Aedes* larva. The HI and CI were 13.08 and 13.28 respectively and the BI was 16.57.

Among the containers, 87% were found to have breeding of *Aedes*. It is interesting to note that *Culex*, *Anopheles* and *Armigeres* were also breeding in containers (Table 1). The most common species of *Aedes* was *Ae. albopictus*. About 86% (60/70) of the clusters were positive for *Ae. albopictus* and 11% (8/60) for *Ae. aegypti*. *Aedes vittatus* larvae were also found in one rural cluster. *Ae. albopictus* was distributed almost equally in rural and urban clusters, whereas 87.5% of the *Ae. aegypti* positive clusters were urban. The distribution of *Ae. aegypti* was,

however, significantly higher ( $p = 0.03$ ) in urban areas (Table 2).

The potential vectors of Japanese encephalitis and filariasis were also found to breed in containers. *Culex vishnui*, *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* were found in 8 (11%), 3 (4%) and 5 (7%) clusters, respectively.

In all, 61 of the clusters were having a high HI and were at high risk of transmitting dengue. A total of 58.5% of areas had a moderate risk of transmission based on the CI, 76% of clusters had BI between 5 and 50, and none of the clusters had a BI of >50 (Table 3). However, several clusters showed a value of >40.

The most common water holding containers found outdoor were of plastic, followed by coconut shells. The breeding preference ratio was highest for tyres (Table 4). The most efficient container in terms of breeding of *Aedes*

Table 1. Genera of mosquitoes identified

Genera	No. of clusters positive (n = 70)	% positive clusters
<i>Aedes</i>	61	87
<i>Culex</i>	15	21
<i>Anopheles</i>	6	9
<i>Armigeres</i>	26	37
Others ( <i>Lutzia fuscans</i> )	2	3

Table 2. Species of *Aedes* identified

Species	Urban (n = 34)	Rural (n = 36)	Chi-square	p-value
<i>Aedes albopictus</i>	29	31	0.06	0.81
<i>Aedes aegypti</i>	7	1	3.8	0.03

(Fischer exact)

Table 3. Risk categorization of clusters according to larval indices

Index type	Category	No. of clusters (n = 70)	%
House index (HI)	<1	9	13
	1–10	18	26
	>10	43	61
Container index (CI)	<10	23	33
	10–30	41	58.5
	>30	6	8.5
Bretau index (BI)	<5	17	24
	5–50	53	76
	>50	–	–

Table 4. Types of outdoor containers

Type of container	Water holding container		Positive container		<i>Aedes</i> (+)ve container		Breeding preference ratio
	n = 2183	% (95% CI)	n = 329	% (95% CI)	n = 290	% (95% CI)	
Plastic container	826	37.84 (37.79–37.88)	87	26.44 (26.17–26.70)	83	28.62 (28.31–28.93)	0.8
Metal container	213	9.76 (9.73–9.79)	43	13.07 (12.87–13.27)	40	13.79 (13.56–14.02)	1.41
Mud pot	230	10.54 (10.51–10.56)	40	12.16 (11.97–12.35)	36	12.41 (12.19–12.63)	1.2
Glass bottle	181	8.29 (8.27–8.31)	32	9.73 (9.55–9.90)	29	10 (9.80–10.20)	1.2
Grinding stone	101	4.63 (4.61–4.65)	30	9.12 (8.95–9.29)	27	9.31 (9.11–9.51)	2
Coconut-shell	443	20.29 (20.25–20.33)	29	8.81 (8.64–8.98)	26	8.97 (8.78–9.16)	0.4
Tarpolin	93	4.26 (4.24–4.28)	25	7.6 (7.44–7.76)	22	7.59 (7.41–7.77)	1.8
Tyre	59	2.7 (2.69–2.71)	22	6.69 (6.54–6.84)	20	6.9 (6.73–7.07)	2.6
Thermocol	37	1.69 (1.68–1.70)	9	2.74 (2.64–2.84)	7	2.41 (2.31–2.51)	1.4

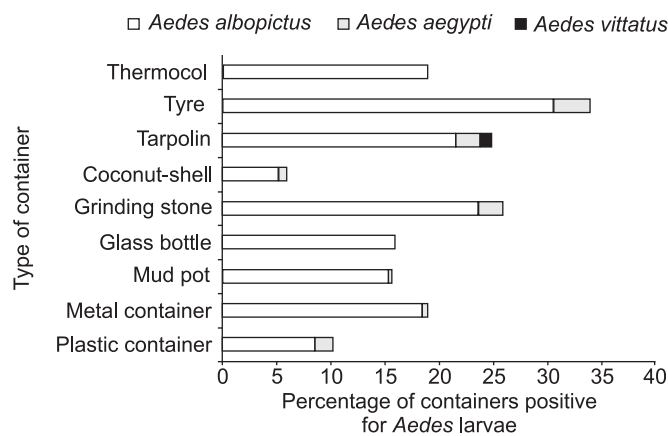


Fig. 1: Different types of containers supporting breeding of different *Aedes* species.

was tyre, followed by grinding stone, tarpolin, thermocol and metal containers (Fig. 1). Breeding of *Ae. albopictus* was found in 30.5% of tyres and *Ae. aegypti* breeding was detected in 3.4% of tyres. Among the grinding stones identified, 23.8% had breeding of *Ae. albopictus* whereas only 2.2% had *Ae. aegypti*. In tarpolin sheets also, breeding of *Ae. albopictus* (found in 21.5%) was more than *Ae. aegypti* (found in 2%). Only 8.6 and 1.5% of plastic containers were positive for *Ae. albopictus* and *Ae. aegypti*, respectively. Indoor positive water holding containers with *Aedes* breeding were few (7.6%—24/314) when

Table 5. Association of container positivity for *Aedes* with spatial and temporal factors

Variable	Total no. of containers (n = 2183)	Container positivity for <i>Aedes</i> (n = 314)	Statistics
Location	Urban (n = 1190) Rural (n = 993)	182 (15.2) 132 (13.2)	Chi-square = 1.8; <i>p</i> = 0.19
Season	Monsoon (n = 1549) Pre-monsoon (n = 634)	197 (12.7) 117 (18.5)	Chi-square = 12.02; <i>p</i> = 0.0005

Figures in parentheses indicate percentages.

compared to outdoor (92.3%—290/314) and included mainly water under flower pots.

There was no significant difference found in container positivity for *Aedes* larvae in urban and rural areas (*p* = 0.19). Significant lesser positivity was found for containers during monsoon (*p* = 0.0005) when compared to pre-monsoon period (Table 5).

## DISCUSSION

The larval survey is the most widely used method for entomological surveillance, for practical reasons when compared to egg, pupal and adult surveys<sup>15</sup>. *Aedes albopictus* was found to be the most common species distributed equally in urban and rural areas, while *Ae. aegypti* was predominantly distributed in urban areas. In semi-arid areas of India, it is documented that *Ae. aegypti* is an urban vector and populations fluctuate with rainfall and other water storage practices. In other countries of South-east Asia Region (SEAR), where the rainfall is >200 cm, *Ae. aegypti* is more stable and distributed in urban, semiurban and rural areas<sup>16</sup>. The average annual rainfall in Thiruvananthapuram is 114.7 cm<sup>17</sup> and the district is located at a relatively low altitude (64 m)<sup>18</sup>. The presence of *Ae. albopictus* in close association with *Ae. aegypti* (sharing same microhabitats) increases the risk for emergence of dengue epidemics. *Aedes albopictus*, being highly adaptive and invasive and flexible in its behaviour, effectively transmits dengue virus even in the absence or insignificant presence of principal vector *Ae. aegypti*, alluding towards its primary, rather than secondary role in the disease transmission. The study on bionomics of this mosquito is necessary, since it is slowly displacing *Ae. aegypti* from its habitats<sup>19–20</sup>. The significant presence of vegetation may be responsible for the abundance of *Ae. albopictus* in the study area. In SEAR, *Ae. aegypti* has been incriminated as the principal epidemic vector, while *Ae. albopictus* has been given the status of secondary

vector, responsible for maintenance of the virus<sup>21</sup>. Despite the frequent isolation of dengue viruses from wild-caught mosquitoes, there is no evidence that *Ae. albopictus* is an important urban vector of dengue, except in a limited number of countries where *Ae. aegypti* is absent, i.e. parts of China, the Seychelles, historically in Japan and most recently in Hawaii<sup>22</sup>. Both *Ae. aegypti* and *Ae. albopictus* are reported to have high vectorial competency, but *Ae. aegypti* has poor vectorial capacity in urban epidemic cycle<sup>15</sup>.

Generally, *Ae. aegypti* is highly adapted to the domestic environment and, therefore, the abundance is positively correlated with increasing urbanization. On the other hand, the distribution of *Ae. albopictus* is associated with vegetation throughout rural and urban areas<sup>23–25</sup>. In Kerala, there is relatively thick vegetation in both urban and rural areas and this may be the reason for the similar distribution of the species in both the areas.

Potential vectors for malaria, Japanese encephalitis and filariasis were also found to breed in containers. There are other studies which report that *An. stephensi* breed in containers including battery shells, tin cans, bitumen drums and tyres<sup>26</sup>. Immature stages of *Culex* also occur in a variety of ground-water habitats, artificial and in natural containers<sup>27</sup>.

The commonly-used larval indices (house, container and breteau) are useful for determining general distribution, seasonal changes and principal larval habitats, as well as for evaluating the environmental sanitation programmes. They have direct relevance to the dynamics of disease transmission. However, the threshold levels of vector infestation that constitute a trigger for dengue transmission are influenced by many factors, including mosquito longevity and immunological status of the human population. There are instances (e.g. in Singapore), where dengue transmission occurred even when the HI was < 2%<sup>28</sup>.

The minimum mosquito density below which arbovirus disease transmission ceases has been debated for many years without a clear resolution. In Singapore, where vector density has been held extremely low through a vigorous control programme DHF/DSS outbreaks still occurred even when the HI dropped to 1%<sup>29</sup>. Since, HI  $\leq$  1% or BI  $\leq$  5% was proposed to prevent yellow fever transmission, these values have also been applied to dengue transmission but without much evidence<sup>30–31</sup>. The high breeding indices for *Aedes* larvae in Thiruvananthapuram district imply their potential for dengue transmission and future outbreaks as in the previous studies<sup>32</sup>.

The most efficient container in terms of breeding of

*Aedes* was found to be tyres followed by tarpolins, grinding stones, etc. Plastic containers and coconut shells were found to be less efficient breeding sites when compared to the above mentioned ones, although these were the most common water holding containers. The breeding sites identified reflect the change in ecology, cultural and social behaviour of population and life style changes<sup>33–34</sup>. Careless dumping of tyres and tubes in and around workshops and retreading centres and up to some extent in peridomestic conditions, mainly in urban setups poses a major problem, as these eventually harbour the mosquito larvae. Tyres were instrumental in the spread of *Ae. albopictus* to the Americas, Australia, Africa and Europe, even displacing *Ae. aegypti* in some areas of America<sup>35–36</sup>. Discarded tyres had a high positivity for *Ae. albopictus* in this study. Tyres as breeding ground for mosquitoes, are not given enough attention; water collected inside is not readily observable. Unlike plastics and coconut shells which are vulnerable to natural and human disturbance, tyres harbour larvae undisturbed and secure. Humidity, cool temperature and reduced light inside it make it an ideal source for *Aedes*. Eggs attached to tyres also play a role in the maintenance of the mosquito population throughout the off season<sup>37</sup>. Tyres have been identified as efficient breeding sites in other studies as well<sup>38</sup>. A similar situation is observed with dumped grinding stones used for grinding cooking ingredients which were used prior to the advent of electric appliances<sup>37</sup>. Indiscriminate use and throw of plastic articles, tarpolins and thermocol remnants are another concern in this regard. Legislations or instructions to regulate these activities may help to reduce the possible mosquito breeding. In other studies rubber plantations where tapping has been suspended, portrayed as an important breeding site in Kerala<sup>39</sup>. In this study, however, not much of such areas have been included as clusters and the focus were on peridomestic and indoor breeding sites.

Other studies showed that *Aedes* breeding is significantly higher in the monsoon and post-monsoon than in pre-monsoon seasons<sup>40–42</sup>. This contrasts with the findings of our study. Probably, the flooding of containers in monsoon may have impeded the breeding of larvae in the monsoon season. It points to the need for intensive control measures in the pre-monsoon season also.

## CONCLUSION

In view of the fact that *Ae. albopictus* was the major species found in the city of Thiruvananthapuram, we recommend further research to isolate virus from this mos-

quito. The expansion of *Ae. albopictus* is significant geographically and its epidemiological significance needs to be worked upon. The high HI and moderately high CI in Thiruvananthapuram restate that the control measures are not proportionate to the need. The reported disease load may also be only the tip of the iceberg considering these indices and there is a need for better active, passive, sentinel and sero-surveillance along with entomological surveillance. Tyre specific legislations and targeted education for those involved in occupations like tyre repair shops are required. Novel methods of control like the use of predators need to be tried in this part of the world. The fact that plastic containers are the most common container is additional health related evidence towards a ban on plastic. Control measures need to be adopted with much vigour even during the pre-monsoon season. More studies on vector breeding preferences and incorporation into regular entomological surveillance are advocated to design targeted education for source reduction activities which are local specific but global implicative for control of dengue.

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Correspondence to: Dr Zinia T. Nujum, Associate Professor, Department of Community Medicine, Government Medical College, Thiruvananthapuram–695 011, Kerala, India.  
E-mail: drzinia@gmail.com

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