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Stegomyia indices of *Aedes* mosquito infestation and container productivity in Alappuzha district Kerala

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Abstract
The aim of the present study is to find out the seasonal variation in container productivity and infestation levels by *Aedes albopictus skuse* and *Aedes aegypti Linnaeus* in Alappuzha district. In total, 1010 houses and 3770 water-holding containers were inspected during both the dry and wet period surveys, and 1606 pupae were collected and all those immature were identified as *Ae. albopictus*. The maximum Breteau and Pupae index was found to be 89.22% and 395% during the month of July. The overall indices were higher in the wet than dry season. Discardable containers were important that had the highest prevalence of *Ae. albopictus* larvae during both the dry and wet periods. Non-discardable containers were important contributors to larval production during the dry period (40%) compared with the wet period (18%). Most of the larval habitats observed in both wet and dry season were small tin and plastic waste containers positive for *Ae. albopictus* larvae. Non-discardable containers were more important contributors for pupal production during the dry period (60%) whereas discardable containers were important pupae producers during wet period (51%). Large and open shape containers produced 71% of total pupae especially plastic sheet. A total of 2514 (146 pools) mosquitoes were screened for dengue and chikungunya virus by RT-PCR. However, there was no detection of dengue and chikungunya virus genomic RNA. Generally, *Ae. aegypti*, (L.) is considered as the primary vector for the spread of dengue and chikungunya virus worldwide. However, our present entomological investigations revealed only the presence of *Ae. albopictus.* So we believe that in Alappuzha district *Ae. albopictus* is the vector for the disease burden of dengue and chikungunya virus.

Keywords: Dengue, Chikungunya**,** *Ae. albopictus,* Breteau, Container and Pupal index

1. Introduction
Aedes aegypti is the primary vector involved in dengue and chikungunya virus transmission [1,2]. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), are two mosquitoes belonging to the *Stegomyia* subgenus, and they are closely associated with peridomestic environments [3]. Several studies have reported that *Ae. albopictus* and *Ae. aegypti* may share the same habitat. Because of this association, it has been hypothesized that some urban areas of Southeast Asia, *Ae. aegypti* has replaced by *Ae. albopictus* [4]. In Kerala dengue is an endemic disease with periodic or annual outbreaks. The history of dengue and chikungunya virus transmission has been documented in Kerala state. Dengue fever was first reported from Kottayam district in 1997 and it spread to other regions of the state later [5]. Since then, dengue cases were reported from the state in a routine manner. *Ae. aegypti* plays a key role in the dengue and chikungunya transmission in various states of India. An epidemic of dengue and chikungunya fever occurred in Kerala where *Ae. albopictus* act as a principal vector in the affected areas [6].
In Alappuzha district, every house has an exposure to open environment with green cover enhances risk of *Ae. albopictus* breeding. Houses are highly clumped within each block and are separated generally by a wooden fence (*Gliricidia sepium* and screw pine (*Pandanus odoratissimus*) and covered by plastic tarpaulin. Almost every house is surrounded by a yard where water holding containers, pine apple and banana plants are frequently found. Alappuzha has a different patterns of dengue transmission in that dengue outbreak will usually start in the early summer (March to April) peak in the monsoon (June to July) and end in the post monsoon. The present study was carried out in Alappuzha district because of its high infestation levels relative to the rest of Kerala state over the previous three years. So, the objective of the present study is to identify breeding habitats of *Ae. albopictus* and *Ae. aegypti* and identify the most prevalent and productive larval habitats and their distribution in wet and dry seasons.

2. Materials and Methods

2.1 Study Site

Alappuzha is an important tourist destination in southern Kerala. It is the smallest district in area but having highest density of population (1504 /Sq.km).The total geographical area is 1414 sq. Km and a population of about 2127789. The rainy season starts in end of May and ends in October. The annual rainfall varies from 1,300 to 1,800 mm rainfall per year. The annual mean temperature is about 26 °C. The average maximum and minimum temperatures are 35.5 and 18.8 °C, respectively.

2.2 Entomological survey

Entomological survey was conducted during wet period (June – 2012 to October-2012) fourteen samplings and the dry period (November 2012 –May- 2013) ten samplings were done. The study area was selected according to the history of high disease incidence, *Aedes* densities, and human population density. All water filled containers were examined for immature stages of *Aedes* species using fine-meshed fishnets. Based on the nature, source and use of the water, positive containers were classified into three main categories: Non discardable, discarded and natural containers as described by Julien *et al.,* 2011 [7]. Larval development sites were noted on: the type of container; its total volume; quality (clear, containing organic matter) of the water; the presence of vegetation around the container, and sun exposure. According to the amount of water in the containers they were classified in to 3 categories: Small: <2 L, Medium: 2 to 50 L, large >50 L. All larvae and pupae from each mosquito positive container were collected, counted and brought to the laboratories and reared until adult emergence for identification using standard keys. Mosquitoes were separated by species and combined into pools with each pool containing a maximum of 50 mosquitoes. Mosquitoes were stored at – 80 °C till use.

2.3 Virus screening

The mosquitoes in each pool were suspended in 1.5 mL of phosphate buffer saline (PBS pH 7) containing 4% bovine serum albumin (BSA) and added 3 glass beads, size of 3.0 to 3.5 mm. The mosquitoes were homogenized in bead beater homogenizer for 60 sec (Biospec. Products Inc. USA). The homogenized mosquitoes were centrifuged at 10000 x g for 10 min at 4 °C. The supernatant was transferred to a fresh tube. Two hundred µl aliquot was used for RNA isolation using Trizol reagent (Invitrogen) according to the manufacturer's instructions and remaining supernatant was stored in -80 ^{0}C until use. Dengue virus was detected from the supernatant using reverse transcriptase PCR (RT-PCR) techniques as described earlier (Lanciotti *et al.* 1992) [8]. Similarly the chikungunya virus was detected as described by Yergolkar *et al.* 2006 [9].

2.4 Statistical analysis

Chi-square test was used to determine the significance of the association between the presence of *Ae. albopictus* larvae in a habitat and habitat type. The significance levels for statistical analyses was at 0.001. Mosquito larval indices such as Container Index (percentage of containers with water and *Ae. albopictus*), House Index (number of positive premises of total number sampled) and Breteau Index (number of positive containers per 100 premises inspected) were calculated.

3. Results

In total, 1010 houses and 3770 water-holding containers were inspected during both surveys, and 1606 pupae of *Aedes* species were collected. All adult specimens that emerged from field collected larvae/pupae were identified as *Ae. albopictus*. In very few habitats *Ae. vittatus* was collected. In the dry season, bucket and utensil containers were most productive for pupae (38%) of *Ae. albopictus*. In contrast to this result, during wet season survey bucket and utensils containers accounted for only 17% of collected *Ae. albopictus* pupae; overall plastic sheet (30.5%) and bucket and utensils (27.5%) were more productive containers than small discarded and other containers. The maximum Breteau and Pupae index was found to be 89.22% and 395% during the month of July which corresponds to the rainy season (Fig 1). The Breteau, house and container index during June to October remain very high. The overall indices were higher in the wet than dry season.

Table 1: Seasonal *Aedes albopictus* larvae and pupae indices in Alappuzha district.

Survey	House index	Container index	Breteau index	Pupae index	Pupae/ container
Dry season	18.16	9.41	25.64	165.56	0.56
Wet season	31.44	12.1	62.56	174.03	0.35

3.1 Container categories

Discardable containers were important containers that had the highest prevalence of *Ae. albopictus* larvae during both the dry and wet periods, with an increasing contribution from the dry period (53% of total larvae positive) to the wet period (59%) ($\chi 2$= 2.07, P >0.07). Non discardable containers were more important contributors to larval production during the dry period (40%) compared with the wet period (18%) ($\chi 2$= 16.99, P <0.001).Natural containers filled by rain had similar relative contributions to larvae collected in the dry and wet periods (6 and 23%, respectively) ($\chi 2$= 16.29, P >0.001). Many of the larval habitats observed in both wet and dry season were small tin and plastic waste containers (34, and 27% respectively), besides many of the habitats positive for *Ae. albopictus* larvae in both the seasons (27 and 30% respectively).

Non discardable containers were more important contributors to pupae production during the dry period (60%) compared with the wet period (37%). Discardable containers were important producers of *Ae. albopictus* pupae during wet period, with an increasing relative contribution from the dry period (38% of total pupae collected) to the wet period (51%). According to the number of *Ae. albopictus* pupae collected, the most productive habitats were those in the plastic sheet (33%), it presented a high number of pupae (No.=296) while not even being a primary abundant container type in the area. A complete listing of containers and infestation rates is presented in Table 2.

Table 2: Seasonal distribution of larvae habitats identified according to habitat type.

Habitat type	Dry season			Wet season		
	Larvae habitats (%)	Habitats with *Aedes albopictus* (%)	*Aedes albopictus* Pupae (%)	Larvae habitats (%)	Habitats with *Aedes albopictus* (%)	*Aedes albopictus* Pupae (%)
Non discardable containers						
Syntex/Drum/Cement tanks	168 (13)	7 (6)	99 (14)	182 (7)	18 (6)	80 (9)
Bowl (ant trap,pet dish etc)	50 (4)	12 (11)	60 (8)	52 (2)	10 (3)	96 (11)
Buckets/Utensils	297 (24)	25 (23)	270 (38)	632 (25)	28 (9)	153 (17)
Discardable containers						
Small tin/ Plastic goods/ Jars/Broken pots	432 (34)	29 (27)	32 (4)	686 (27)	92 (30)	91 (10)
Tire	83 (7)	7 (6)	46 (6)	41 (2)	15 (5)	67 (8)
Plastic sheet	121 (10)	22 (20)	199 (28)	395 (16)	74 (24)	296 (33)
Natural containers						
Coconut/Coco shell	94 (7)	4 (4)	5 (1)	412 (16)	44 (14)	42 (5)
Bamboo stumps/Tree holes/Leaf axils	12 (1)	2 (2)	5 (1)	113 (5)	29 (9)	65 (7)
Total	1257	108	716	2513	310	890

Large and open shape containers, but in the shade, produced 71% of total pupae especially plastic sheet. Moreover, 61% of pupae were collected in containers with organic pollution, 70% and 30% in open and partially open shape containers respectively (Table 3). Containers types are grouped according to capacity categories, 32.25% had a capacity >1 L, 55.60% a capacity of 1-25L, and 12.14% <25L, those contribution to the number of pupae.

Table 3: Percentage of water holding containers of each type and of pupae collected container index, and pupae per container for every variable measured and every type of container.

Larval habitats		A	B	C	D	E	F	G	H	I	Total	%Pupae collected	Container Index	Pupae container
Water with organic pollution	**F**	59	69	50	53	26	60	58	100	87	60	61	16.59	0.43
	N	41	31	50	47	74	40	42	0	13	40	39	02.84	0.40
Sun exposure	**F**	79	97	55	54	16	33	87	22	93	52	71	16.50	0.59
	N	21	3	45	46	84	67	13	78	7	48	29	05.27	0.24
Lid status	**P**	47	21	10	0	80	0	0	0	0	10	30	11.73	1.20
	N	54	79	90	100	20	100	100	100	100	90	70	11.00	0.34
Container searched	**S**	1	98	9	93	19	80	12	100	98	59	32	11.60	0.23
	M	21	2	85	7	81	19	9	0	2	30	56	8.22	0.78
	L	78	0	6	0	0	1	80	0	0	11	12	16.45	0.49

A- Syntex/Drum/Cement tank/Trough ,B- Bowl (ant trap, pet dish etc), C- Buckets/Utensils, D- Small Tin/plastic wastes/Broken bottles/jars/pots, E- Rubber tire, F- Plastic sheet/Roof gutter, G- Rock & mud pools, H- Bamboo stumps/tree-holes/Leaf, I- Coconut/coco shell. F- Fully, N- Not, S – Small, M- Medium, L – Large,

3.2 Rain fall

Container productivity examined in relation to monthly rainfall. The relationship of increasing larval population (BI) with rainfall is shown in Fig.1, where the seasonal pattern of *Ae. albopictus* was fairly close to variations in rainfall. The heaviest rainfall occurring from May to August resulted in higher BI, in those months. The maximum Breteau index peak obtained was 89 corresponding to the heaviest rainfall in the month of June (334 mm).

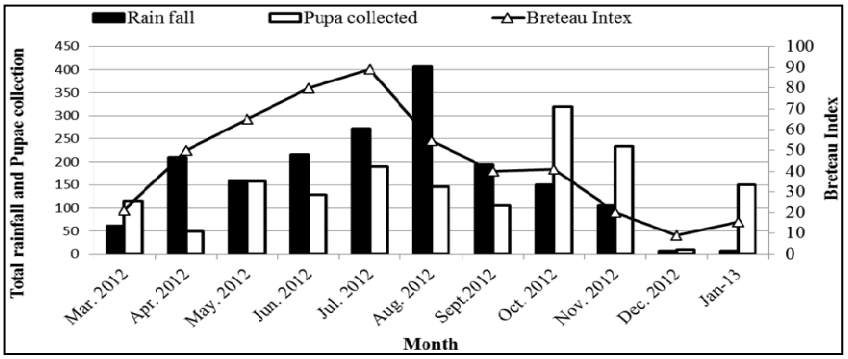

Fig 1: The relationship of increasing larval population (BI) with rainfall

3.3 Viral screening

A total of 2514 (146 pools) mosquitoes were screened for dengue and chikungunya virus by RT-PCR, of which 2487 were *Ae. albopictus* (141 pools) and 27 *Ae. vittatus* (5 pools).

However, there was no detection of dengue and chikungunya virus genomic RNA.

Table 4: Chikungunya and Dengue virus serotypes detected by RT-PCR in field-caught *Aedes* mosquitoes pools (number of mosquitoes) from Alappuzha district

Mosquitoes	Dry season	Wet season	Total	Pools positive
Aedes albopictus	14 (157)	127(2330)	141 (2487)	0
Aedes vittatus	0	5(27)	5 (27)	0
Total	14 (157)	132 (1802)	146 (2514)	0

4. Discussion

Alappuzha is the one of the district that has been greatly affected by dengue and chikungunya virus. Ever since dengue cases were reported in 1997[5], the local authorities in Alappuzha district have been battling the disease with the use of insecticides, larvicides as well as education and community involvement in the removal of artificial containers that serve as the larval habitat. Even though mosquito control efforts have been ongoing, results from this study show that larval habitats are still common in Alappuzha district and *Ae. albopictus* larval indices are high enough to maintain dengue transmission, especially in the wet season [10]. According to our surveys, water storage in Alappuzha in containers like buckets and plastic can (50 L capacity) used to store water become common larval habitats in areas where availability of piped water is a problem [11]. These bucket and plastic can and large containers can hold enough water during the dry season and serve as productive mosquito larval habitats. Among natural larval habitats sampled, coconut shells were the most common natural larval habitats encountered in all areas because coconut constitutes a staple food for local population. During the summer, rainfall can easily reach 143 mm/month and neglected water containers like plastic sheet, can, and other small plastic waste collects rainwater, thus these containers provide larval habitat and vector population size by creating habitat during dry season. Apparently, enough water to support production of immature accumulates in containers of these types even during the dry season when rainfall is sporadic [12].

Pupae distribution is varied in different containers according to the dry and wet season. Surprisingly, plastic sheet and buckets could be indicated as key containers in both seasons. Plastic sheet, are the unique container type classified as key container in our study. Abundant containers, like broken bottles, cans, and plastic goods had low production. With the application of pupal survey, large containers have been considered to account for most adult *Ae. albopictus* in some areas [13]. However this was not the case in this district, where differences in productivity between large and medium containers accounted for more number of pupae collected. A previous study has shown detection of chikungunya virus from *Ae. albopictus* in Kerala [14]. In our survey, no mosquito infections were detected, however, dengue and chikungunya incidence in Kerala has shown growing trend in the last decade. *Ae. aegypti* is regarded as the primary vector for chikungunya and dengue virus. However, survey carried out in Lakshadweep islands and La Reunion Island in the Indian Ocean, *Ae. albopictus* is now considered to be the main vector of dengue and chikungunya viruses and absence of *Ae. aegypti* [15]. Similarly, in the present survey could not detect *Ae. aegypti* during house hold survey, which suggests the possibility of the role of *Ae. albopictus* as the vector of dengue and chikungunya virus in Alappuzha district.

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