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Dengue vectors prevalence and the related risk factors involved in the transmission of dengue in Thiruvananthapuram district, Kerala, south India

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ABSTRACT

Background & objectives: A longitudinal, entomological and virological study was conducted from 2007 to 2010 in four dengue fever affected areas of Thiruvananthapuram district, Kerala to understand the risk factors involved in the dengue transmission.

Methods: Aedes surveys were carried out seasonally in the selected localities both indoors and peridomestic sites. Water holding containers were sampled for the presence of immature. Outdoor and indoor resting/landing mosquitoes were collected. Blood meal identification was performed by gel diffusion test and viral assay using the ELISA test.

Results: The species found were *Aedes (Stegomyia) aegypti* (Linn.), *Ae. (Stegomyia) albopictus* (Skuse) and *Ae. (Stegomyia) vittatus* (Bigot). *Aedes aegypti* and *Ae. albopictus* immature stages were also found during the study period. *Aedes aegypti* was the only prevalent species in the water-starved Vizhinjam, a rural coastal area with breteau index (BI) ranging from 40 to 271. *Aedes albopictus* was recorded in rest of the three surveyed localities—two urban and one rural ghat areas of Thiruvananthapuram district.

Interpretation & conclusion: The vector control measures should be focused mainly on source reduction of water storage containers present in both outdoor (*Ae. albopictus* and *Ae. vittatus*) and indoor (*Ae. aegypti*). To achieve effective vector management, a public health response beyond routine larviciding or focal spraying is essential throughout the year.

Key words *Aedes aegypti; Ae. albopictus;* dengue; India; Kerala

INTRODUCTION

Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are major public health problems with almost one half of the global human population at risk[1]. Dengue is one of the most widespread infectious diseases globally and its transmission now occurs in 128 countries[2]. The Western Ghats region, earlier infrequently encountering sporadic cases of dengue infection, is now reported to be suddenly experiencing a string of focal outbreaks in different districts from 2003 onwards[3]. After an epidemic in Kerala in 2003, enormous number of cases were reported from several districts particularly those in the sylvan environs of the Western Ghats such as Iduki and Kottayam districts[3–5]. During the year 2006, 65% of the total cases were reported from Thiruvananthapuram district, Kerala state. *Aedes albopictus* has been considered a potential vector of dengue and several virus isolations have been made in Southeast Asia[6] and in India isolation of DENV had been documented only once in the east[7]. Therefore, this study was undertaken to understand the risk factors involved in the epidemiology of dengue in this area despite more cases are reported to comprehend the dynamics of dengue transmission with particular reference to breeding habitats, abundance and virus infection rates of different dengue vector species present both in domestic and peridomestic areas.

MATERIAL & METHODS

Study area

Kerala is an Indian state with a total area of 38,863 km^2 and population of 31,838,619. The latitude and longitude are 8°18' N and 74° 52' E to 77° 25' E, respectively. Frequent outbreaks of DF have recently been documented in Kerala, southern India[8]. Thiruvananthapuram region is fairly humid and warm throughout the year with relative humidity and temperature ranging from 70–90% and 22–34.5°C, respectively. The annual precipitation is high reaching up to 300 cm, with maximum number of rainy days (18–21 rainy days/month) being in May to August (Meteorological Department, Meteorological

Centre, Thiruvananthapuram). The study area is typically dry for one to two months from January to March. From 2003 onwards, more number of dengue cases were reported from Thiruvananthapuram district and the area received heavy rainfall from the month of June to September (southwest monsoon), and some rains from October to November under the influence of northeast monsoon. Four distinct seasons are observed in this region and the field surveys were undertaken during these seasons only. These are: (i) post-monsoon season (January–March); (ii) summer season (April–June); (iii) southwest monsoon season (July–September); and (iv) northeast monsoon season (October–December).

In Thiruvananthapuram district, dengue incidence per one lakh population was >2 and during the year 2006 about 65% of the dengue cases in Kerala were reported from Thiruvananthapuram district. Thus, the study sites were selected purely based on the dengue case incidences. Based on the dengue cases reported during 2006 from each locality two rural and two urban localities were selected for this study. Vizhinjam Panchayat (Kottapuram) is a rural costal area situated 15 km from Thiruvananthapuram with cases of dengue (11–15 cases). Vellanadu PHC (Puthukulangara) is again a rural ghat area situated 15 km from Thiruvananthapuram reporting 16–22 dengue cases. Medical college area (Rajiv Gandhi Nagar—Ward No. 12) is an urban locality situated 6 km from Thycaud area reported with 11–15 cases. Nemam (Karikkamandapam—Ward No. 52) is an urban area reported with 13 dengue cases. All these localities were selected based on the confirmed reports of dengue cases (DHS, Kerala).

Entomological surveillance

Aedes surveys were carried out in 30 houses (both indoor and peridomestic sites) from the peridomestic man-made breeding habitats like cement cisterns, cement tanks, metal containers, plastic drums, plastic containers, metal drums, grinding stones, mud pots, bottles, discarded containers, flowerpots, flower vases, tyres, water pumps, latex cups, polythene sheets, flowerpot trays and also the natural breeding sites like coconut shells, tree-holes, plant axils, coconut leaf-thatched sheets, fallen spathes or bracts (deciduous bracts that envelop or surround the fluorescence or flower) of a coconut palm systematically and reared individually[9–10] from these selected localities seasonally.

Every accessible water-holding container in and around the house was sampled for the presence of immature mosquitoes. Small containers (<20 litre capacity) were completely drained through a steiner into a white larval sampling tray (25 × 20 × 4 cm) to collect larvae and pupae. Larger containers were sampled using a 250 ml larval dipper. Five dips were taken from the surface water of each container (four dips evenly spaced around the edges of the container and one at the centre). All the larvae and pupae were brought to the field laboratory in labeled containers. Every water-holding container was categorized according to the type of container, container function, shape, maximum capacity, volume of water in the container, and material and presence of a cover. Breteau index (BI), house index (HI), container index (CI) and pupal index (PI) were calculated[11]. In the laboratory, III and IV instar larvae and all pupae were transferred to holding containers which were covered with permeable gauze. From March 2007 to March 2010, each larva was individually reared and identified at the adult stage[12].

Adult survey

In the outdoor settings, wild adult mosquitoes were collected while resting or landing for 15 min per house using the mouth aspirator and flash light. In each area, per survey, two insect collectors spent 2 h each (four man hour per village), and the average number of adults per man hour (PMH) was estimated. *Aedes* species landing on human volunteers (from whom informed consent was obtained) were collected in the morning and late afternoon for 30 min per volunteer, and the density was expressed as female landing PMH. Adult mosquitoes were morphologically identified, separated by sex, pooled and stored in liquid nitrogen. The remaining specimens were held in the field laboratory for 24–48 h for digestion of their blood meal, subsequently pooled (pool size ranged from 1 to 11 females), stored in liquid nitrogen and transferred to the laboratory for viral assay[13] using the ELISA test. Institutional Ethical Committee clearance was obtained before the initiation of this study. A sample of blood engorged females was used for the host blood meal identification by gel diffusion test[14].

Statistical analysis

Descriptive statistics of mean and confidence interval was used to calculate the larval indices. Significance of larval indices in different areas was compared by using ANOVA. Paired sample *t*-test was used to compare significance of indoor and outdoor positive habitats. Pearson's correlation was used to find out any relation between rainfall and PMH density for indoor and outdoor collections. ANOVA, correlation and *t*-test were carried out using the SPSS version 16.0 software package.

RESULTS

Distribution of Aedes immature mosquitoes among containers and households

During the study, the distribution of larvae and pupae was over-dispersed with much greater mosquito densities in some households than others in the water-holding containers and most of the containers had few or no aquatic stages. *Aedes aegypti*, *Ae. albopictus* and *Ae. vittatus* were the species present in the studied localities. The main source of breeding in the peridomestic habitat was cement tanks, mud pots, metal/plastic containers and discarded containers. The main source of breeding in the indoor habitats was cement tanks, metal containers and plastic containers. *Aedes aegypti* was the only species found in Vizhinjam and *Ae. albopictus* was found breeding in the Nemam (urban) and Vellanadu (rural) areas. Several breeding grounds of different *Aedes* species are mentioned in Table 1. *Aedes albopictus* breeding was observed in the cement tanks, plastic containers, metal containers, ant guards, latex cups, tree-holes, banana plant leaf axils, mud pots, flowerpot trays, grinding stones, tyres, glass bottles, spathes or bracts and discarded containers. Cement tanks, plastic containers, metal containers, tyres and flowerpots showed *Ae. vittatus* breeding. Dengue vector has also been found breeding in cut bamboos, plastic bags and GI sheets used to cover houses[15]. Mixed breeding of *Ae. aegypti* and *Ae. albopictus* was recorded from the habitats like cement tanks, metal containers, tyres, flowerpots, discarded containers, plastic containers and tree-holes. In addition to this, mixed breeding of *Ae. aegypti* and *Cx. gelidus* was reported from the tyres and the combination of *Ae. albopictus* and *Ae. vittatus* was also observed in cement tanks. Besides this, simultaneous occurrence was also recorded for *Ae. albopictus* and *Heizmannia greeni* from tree-holes and *Ae. aegypti* and *Cx.(Lut) fuscanus* from cement tanks.

BI, HI, CI and PI indices are simple to adopt and logistically better and thus these values employed for the comparative analysis[16], indicated the comparison of larval indices in four villages of Thiruvananthapuram district of Kerala (Table 2). The results showed that HI and BI have high significant differences (p <0.001), whereas CI and PI showed significant difference (p <0.05) in villages. Table 2 indicates that Vizhinjam got high HI, CI, BI, and PI values when compared to other villages. HI, CI, BI and PI values of Nemam, Vellanadu and Rajiv Gandhi Nagar villages showed no significant differences (p >0.05), but HI, BI and PI values of Nemam, Vellanadu and Rajiv Gandhi Nagar villages varied only with Vizhinjam (p <0.05), CI value of Vizhinjam and Vellanadu not significantly varied (p >0.05) but Nemam and Rajiv Gandhi Nagar have significant differences (p <0.05) in Kottapuram. Larval indices like HI, CI, BI and PI gradually increased after the rainfall.

Adult indoor resting collection of *Ae. aegypti* was recorded from Vizhinjam area only. In the outdoor landing collection, *Ae. albopictus* was only recorded in Vellanadu and Nemam areas. In the Medical College area, both *Ae. albopictus* and *Ae. vittatus* were collected. In the indoor landing collection, only *Ae. aegypti* was collected from Vizhinjam. Thus, *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* were the prevalent species from this district. Of all the four different localities surveyed, a rural coastal

Table 1. Breeding habitats observed for different *Aedes* species of mosquitoes

Species	Breeding habitats
Ae. aegypti	Cement tanks, plastic containers, metal containers, coconut shells, mud pots, tyres, discarded containers, plastic drums, flowerpots and fallen spathes or bracts.
Ae. albopictus	Cement tanks, plastic containers, metal containers, ant guards, latex cups, tree-holes, banana plant leaf axils, mud pots, flowerpot trays, grinding stones, tyres, glass bottles, spathes or bracts and discarded containers.
Ae. vittatus	Cement tanks, plastic containers, metal containers, tyres and flowerpots.

Table 2. Comparison of larval indices in different areas in Thiruvananthapuram district, Kerala (March 2007 to March 2010)

Larval indices/ Village	Kottapuram (Vilingam panchayat)‡	Karikkamandapam (Nemam)‡	Vellanad (Puthukulangara)‡	Medical College (Rajiv Gandhi Nagar)‡	p-value
House index (HI)	62.8 (55.2–70.4)	24.2 (13.1–35.4)	26.7 (14.9–38.4)	21.7 (10.9–32.4)	15.9 (0)†
Container index (CI)	33.3 (23.0–43.6)	17.5 (8.4–26.6)	21.4 (9.5–33.2)	14.5 (8.1–20.9)	3.4 (0.024)*
Breteau index (BI)	129.8 (97.9–161.7)	35.8 (16.9–54.8)	46.0 (20.5–71.5)	34.4 (14.8–54.1)	16.0 (0)†
Pupal index (PI)	192.7 (55.5–329.9)	35.3 (–3.9–74.6)	40.1 (2.8–77.4)	40.1 (2.3–78.0)	4.7 (0.005)*

†Significant at 0.1% level (p <0.001); *Significant at 5% level (p <0.05); ‡Mean (95% CI).

area Vizhinjam harbored only *Ae. aegypti* and Vellanadu (Rural) area reported only *Ae. albopictus* whereas the two species *Ae. albopictus* and *Ae. vittatus* were harboured in Nemam (Urban) and Medical College area. No correlation ($r = 0.046$) was observed between rainfall and PMH density for the indoor resting collection and outdoor landing collection ($r = -0.023$).

Blood feeding pattern of these mosquitoes showed 76.9, 75 and 33.3% human feeding for *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* mosquitoes, respectively. Mosquito pools collected from Thiruvananthapuram district, Kerala was found positive for DEN virus in one pool of *Ae. aegypti* (four females) collected from Vizhinjam area during indoor resting collection during November 2007. Similarly, two pools of *Ae. aegypti* collected from Vizhinjam area during September and December 2009 from an indoor resting collection were found positive. Only three pools of *Ae. aegypti* collected during the indoor resting collection between November 2007 and December 2009 were found positive for DENV. In our study, there was no DENV infection from *Ae. albopictus* and *Ae. vittatus*.

DISCUSSION

Kerala started reporting dengue cases regularly after the 2003 epidemic. Based on 2006 dengue cases reported in Kerala, an epidemiological surveillance was initiated in the dengue reported areas of Thiruvananthapuram district to find out the various factors involved in the transmission of dengue. Larval indices were used to quantify vector breeding sites, and to identify productive water container types as well as measurement of the adult vector abundance. Greater knowledge about dengue and its transmission was associated with mosquito breeding and production. All the three species of *Aedes* mosquitoes were found in Thiruvananthapuram district and *Ae. albopictus* was the most prevalent and widely distributed. *Aedes aegypti* was localized in a rural coastal area at Vizhinjam where water scarcity is recorded.

Most productive habitats present in both outdoor (cement tanks, mud pots, metal/plastic containers and discarded containers) and indoor (cement tanks, metal containers and plastic containers) habitats were recorded with immature breeding of vector mosquitoes observed continuously throughout the year and required more attention for source reduction activities. These habitats were perennial breeding sites and prevalent in those areas. Breeding percentage present in the containers in both outdoors and indoors did not show any significant difference ($t = 0.950$, $p > 0.05$) and hence, control operation with source reduction should be concentrated both in outdoors and indoors with equal concentration. Maximum number of containers like cement tanks, plastic containers and metal containers, breeding mainly during the epidemic months of June, July, September and November must be included for the control operation like source reduction. Dengue cases reported in Kerala showed that two peaks coincided with the monsoon periods (Fig. 1) during that time period maximum containers showed immature breeding of the *Aedes* mosquito vectors (Fig. 2) and required to be covered tightly under the vector control operation. *Aedes albopictus* was more dependent on rainfall compared with *Ae. aegypti* and its larval density sharply increased after monsoon rains which filled up all the peridomestic containers strewn around that area[17]. All these risk factors found in the breeding of *Ae. aegypti*

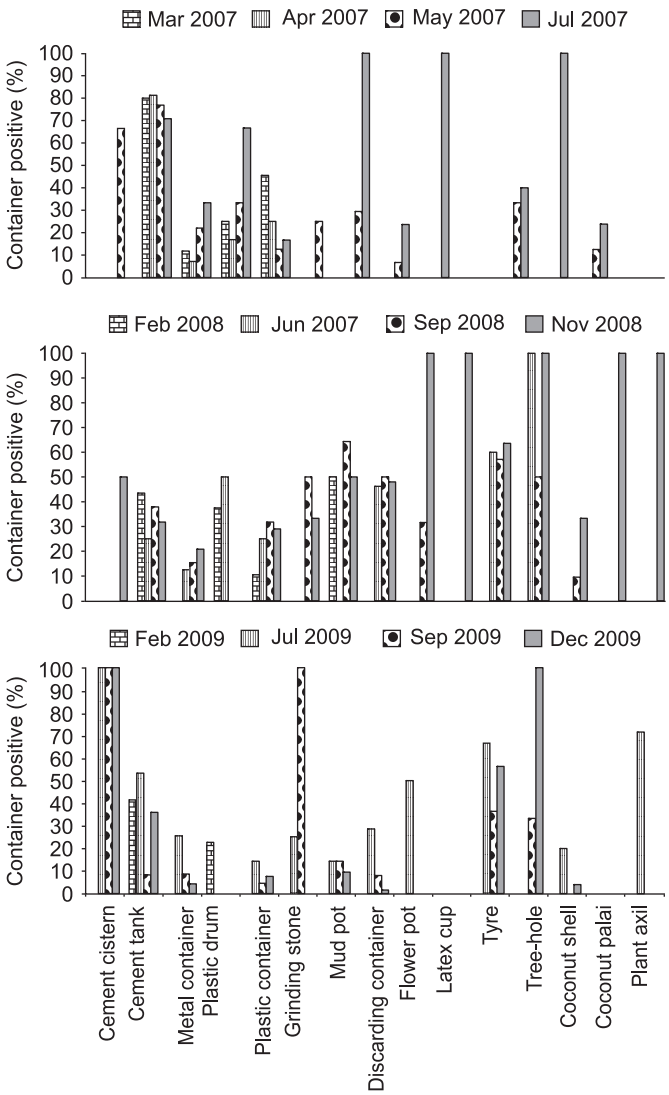

Fig. 1: Comparison of maximum number of containers breeding during different periods in Thiruvananthapuram district, Kerala.

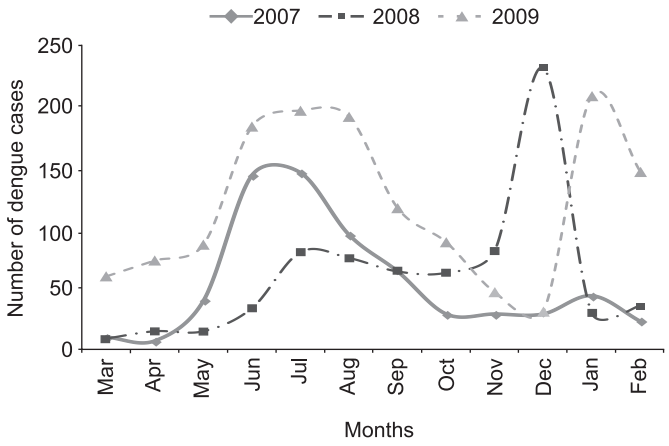

Fig. 2: Month-wise dengue cases reported in Kerala state, India (2007–2009).

and *Ae. albopictus* suggest that targeting specific types of water-holding containers would enable a more focused approach for vector control than attempting to eliminate all water-holding containers[18–19].

Epidemic of DF was mainly due to the mud pots breeding used as water storage containers that supported *Ae. aegypti* breeding[20]. In Maharashtra[21], Samui Island[22] and Dominican Republic[23], infestation of *Ae. aegypti* was also mainly due to the breeding in cement containers. However, in western India (Rajasthan), breeding was mainly in mud pots[24] as we observed in some households. Chennai coastal area mostly breeding outdoors was also similar to Vizhinjam area breeding mainly the *Ae. aegypti* mosquitoes[18]. But here we found *Ae. aegypti* breeding mainly in the cement tanks, metal/plastic containers and discarded containers. The cement tanks could not be drained out completely and remained a perennial source of breeding throughout the year due to lack of proper outlets and, therefore, retained small quantities of water sufficient for immature stages to thrive. Even though *Ae. aegypti* was strongly associated with urban environments, it was found near the rural coastal area. This study showed that *Aedes* species have adapted to breed in a variety of man-made and disused containers in urban/rural areas in addition to its natural breeding sites.

The number of dual infestations was still significantly higher than the expected. Mixed infestation can probably be attributed to similar preferences of the two species for oviposition. It has been reported that *Ae. aegypti* is more likely to oviposit in containers already inhabited by larvae and pupae of conspecifics, and the gains achieved through targeted control of productive containers would be short-lived if females were diverted to oviposit in alternative sites. The results described here also indicate that containers could be covered to prevent access by females seeking an oviposition site[18, 25–27]. Larval indices showed significant differences between villages except in Vizhinjam which had higher indices value and bred only *Ae. aegypti*.

Except from the rural coastal area, *Ae. albopictus* dominated the collections from urban areas and rural ghat region. In Taiwan, the northern limit of *Ae. aegypti* appeared to be restricted by low temperatures that *Ae. albopictus* was better able to tolerate[28]. This study also revealed that the *Ae. albopictus* was only present in the rural ghat sections and also distributed in the urban areas. The difference in the temperature may provide a plausible explanation for the absence of *Ae. aegypti* from the other areas. Among the three *Aedes* species recorded, only *Ae. aegypti* was always predominant species found in the Vizhinjam area. This mosquito was found biting humans throughout the year by its well-known anthropophilic nature[29]. *Aedes albopictus* was prevalent mainly during rainy season preferring to breed outdoors in discarded containers, an observation similar to that made by Hawley[6]. In Malaysia and Thailand[30–31] *Ae. albopictus* was the primary vector for recent outbreaks of chikungunya. *Aedes albopictus* exhibited a preference for oviposition in container habitats and associated more with peri-urban and rural environments[32–34].

The predominance of *Ae. albopictus* and the total absence of *Ae. aegypti* in other places, could possibly be acting as a secondary vector. *Aedes albopictus* feed readily on humans and animals and are more likely to feed outdoors as compared to with *Ae. aegypti*[17, 35]. *Aedes vittatus* was found breeding during the monsoon season only and a very few adults were captured in human landing catches. Similar studies in the villages around Vellore town showed the isolation of all the four serotypes from mosquito samples collected which were demonstrated during 1968[36].

All the DENV positives were from three pools of *Ae. aegypti* collected from Vizhinjam area obtained from indoor resting collection indicating that this species was the primary vector of dengue in these places. During epidemics in Maharashtra[21], and Gujarat[37–38] specimens examined were found infected with DENV as observed in Singapore[39]and Senegal[40]. *Aedes vittatus* appears to be playing no role in dengue transmission in these areas because of its poor anthropophilic nature and no isolation of virus from a very few adult specimens obtained in the field. A continued vigilance and monitoring of *Aedes* mosquito species composition in a particular place before the dengue vector control operation is highly encouraged. This suggestion is particularly important because low levels of past infection with DENV are reported from this highly

susceptible vector population[41]. Development of an *Aedes* vector control programme is recommended to prevent the establishment of *Ae. aegypti* and to control *Ae. albopictus*, which is a secondary vector of DENV, as well as a vector of CHIKV[42]. A complex association was found between water supply, or larval breeding and higher vector abundance in Vizhinjam area.

Draining water from cement tanks once in a week would be an ideal and effective way to kill larvae and pupae of *Ae. aegypti* because the generation time from larva to adult takes two or more weeks. Elimination of breeding in these tanks could reduce *Ae. aegypti* pupal populations by approximately one-third, leading to reduced adult population size and risk of disease transmission. Covering water-holding containers should also reduce the risk of breeding by preventing female mosquito's access to water in which these oviposit. Vector control efforts for this species should focus primarily in these types of coastal rural land areas[19].

Aedes albopictus breeding was more in the peridomestic habitats as observed in these areas. Therefore, targeted control of this habitat types could contribute to large reductions in *Ae. albopictus* populations in these areas. Containers that are in frequent use for hygiene, cooking, and drinking purposes are less likely to become breeding sites than long-term water-storage containers. In a similar vein, containers that have been discarded and are not in active use are much more likely to be colonized by *Ae. albopictus* than those containers specifically used to store water. Elimination of breeding habitat in discarded containers should be a priority for *Ae. albopictus* vector control. This investigation identified *Ae. albopictus* in the study villages. Thus, the potential risk for DENV and CHIKV transmission is greater throughout this region.

Aedes aegypti and *Ae. albopictus* seem to be restricted to their own limited breeding territorial sites before and after the onset of monsoon, so control measures focusing on prime breeding sites should be more labour-intensive for routine larvicide application and source reduction or elimination. Due to the absence of effective vaccine, the source reduction is the main control strategy practised everywhere. After the proposed Vizhinjam harbour construction is completed, fresh piped-water supply from Vellayani lake near Vizhinjam area will be supplied to these localities which will change the entire dengue scenario and bring down the dengue cases. Based upon the present observations equal attention for the control operation with the help of local community should be given to the outdoor and the indoor key productive containers. Covering water containers tightly is very effective against vector breeding only if the cover offers full protection. Close interaction between communities and municipal vector control services is crucial for the success of dengue vector control. Moreover, these aspects indicate for an urgent need for taking up disease/vector surveillance to know in detail about the impending epidemic to devise appropriate dengue vector control in Kerala state. The effective vector control measures can substantially reduce larval and pupal counts and *Aedes* breeding in these areas, to bring down the disease burden.

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