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Aedes mosquito survey within the premises of University of Sri Jayewardenepura to determine its population density and seasonal shift in relative abundance.

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Abstract

The Premises of the University of Sri Jayewardenepura was surveyed in the month of May for possible breeding habitats of mosquitoes which were mapped out and the immature stages of collected were identified. Subsequently investigations were made by placing ovitraps within the University premises from June to November 2008, to determine the abundance of *Aedes aegypti* and *Aedes albopictus* detected during the previous survey in the month of May. An *Ae.aegypti* positive container was detected only once in all the indoor and outdoor container observed and no *Ae.aegypti* positive ovitrap was recorded during the study period.. The Ovitrap Index (OI) for *Ae.albopictus* was calculated monthly. Breeding of *Ae.albopictus* was found in both indoor (Mean Monthly OI =13.3) and out door containers (Mean Monthly OI 35.3). During the dry season the rainfall recorded a monthly range of 45.6 mm to 152.6mm. The larval population of *Ae.albopictus* remained low during this period with an OI range from 0 to 12.5. However, during the rainy season which showed a monthly range of 294.9mm to 414.4mm, the OI increased exponentionally (OI ranges from 13.3-80). In the month of September, *Ae.albopictus* were recorded in indoor ovitraps too (OI=13.3), indicating its ability to replace the breeding places of *Ae.aegypti,* under certain environmental conditions. The monthly dengue incidence reported from the neighboring area declined in the month of August and started to increase again in the month of September. The ecological conditions in theses areas are very much similar to those within the University premises. The OI values of dengue vector mosquito species could be utilized to indicate a potential increase in the dengue incidence.

Key words: *Aedes albopictus*, Dengue Fever, Ovitrap Index, Mosquito breeding habitats, Integrated Vector Control

Introduction

Dengue fever and the severe forms –Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) have emerged as the most important vector borne disease in Sri Lanka in the past few years. At present, dengue is endemic and is an important public health problem in the country. The disease is found mainly in urban and sub-urban areas. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) have been involved in the transmission of classical dengue fever (DF) and dengue haemorrhagic fever (DHF) in many urban areas of South –East Asia (Hammon, 1966). In the year of 2009, there were 35007 reported dengue cases with 346 deaths in the country (Epidemiological Unit, Ministry of Health, Nutrition and Welfare, Colombo). Dengue epidemics are an indication of the failure of public health systems to respond rapidly (WHO,2008) and appropriately to prevent such an occurrence in Sri Lanka. The increasing burden of dengue in Sri Lanka is a matter of serious concern since not only the disease is spreading to new areas of the country, but also to other areas of the region. This may have an adverse impact on tourism and other industries resulting in heavy economic losses to a country like Sri Lanka.

The container inhabiting mosquito species such as *Ae.aegypti* and *Ae.albopictus* are becoming most important with the rapid frequency of epidemic DF and DHF. The primary vector of DF in Sri Lanka is *Ae.aegypti* while *Ae. albopictus* is the major secondary vector. *Ae.vittatus* and *Ae.polynesiensis* also act as secondary vectors. These secondary vectors are equally important as the primary vector in the maintenance of the virus. Dengue virus serotype 3 was isolated from wild caught adult females of *Ae.albopictus* (Hapugoda et al, 2004). It is believed that this species acts as a reservoir of this disease. However the role of *Ae.albopictus* is not properly understood yet (Hapugoda, 2004).

Both *Ae.aegypti* and *Ae.albopictus* are highly anthropophilic day time biters and they are dominant in domestic and peridomestc environments. *Ae.aegypti* usually breeds in indoor artificial containers. *Ae.aegypti* is an urban mosquito species while *Ae.albopictus* prefers semi urban environments and breeds in outdoor natural breeding sites. It also breeds in indoor and outdoor artificial containers (Hoedojo and Suoro, 1990)

This study was primarily carried out to formulate suitable recommendations and suggestions based on the findings to minimize *Aedes* mosquito breeding places within the university premises of Sri Jayewardenepura, where around 4000 people including academic, supporting staff and students are present during day time, which coincides with *Aedes* biting time. It is believed that infection may occur very quickly and spread out fast to other neighboring areas from places such as universities, schools, garment factories etc. Simultaneously a comparison of Ovitrap Index (OI) of *Ae.albopictus* with DF cases reported in areas which have similar ecological and climatic conditions and their temporal association with the rainfall are presented which would enable to detect a potential dengue outbreak situation.

Materials and methods

Study period

This study was conducted from May to December 2008 within the University of Sri Jayewardenepura (Figure1). It is located approximately 15 km away from the Colombo capital, in an area of approximately 25acres^2 where an average of 4000 student and staff population is frequently present. It comes under Maharagama Municipal Council, bounded by Piliyandala and Boralesgamuwa Pradesiya Sabhas. These are considered as dengue endemic areas (Epidemiological Unit, Ministry of Health, Nutrition and Social Welfare Colombo). The study site is located in a semi urban area, with natural vegetation and artificially cultivated home gardens.

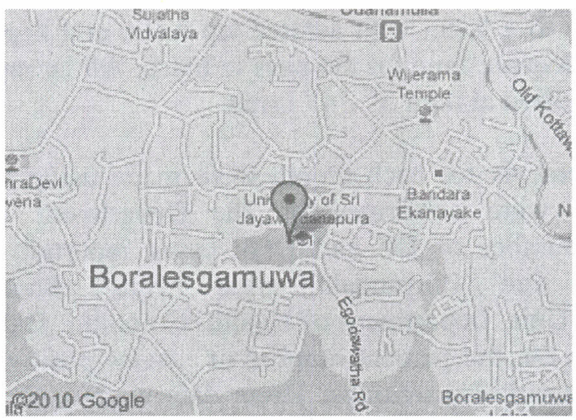


Figure 1. Site Map of University of Sri Jayewardenepura
(Source: Google earth,2009)

Larval surveillance

A preliminary survey was carried out prior to the ovitrap survey, to detect potential breeding habitats of mosquitoes and to determine whether they harbour *Aedes* mosquito immature stages. Special attention was focused on areas where water gets accumulated throughout the year. All larvae or pupae were collected in each positive breeding place (natural or artificial). Larvae and pupae were taken using ladles and pipettes, placed in to vials, labeled and bought to the laboratory, where they were identified using standard taxonomic keys.

Water collected places within the University premises were marked with the GPS points and *Aedes* positive sites were mapped out for launching source reduction method by the University cleaning service.

Ovitrap survey

Ovitrap surveys were carried out for a six month of period randomly .

Each ovitrap was a 300 ml plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter was 6.5 cm and the container was 9.0 cm in height. The outer wall of the container was coated with a layer of black oil paint. An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally in each ovitrap which was filled with dechlorinated tap water to a level of 5.5 cm.

Thirty ovitraps were placed both in doors and out doors fortnightingly. They were collected and checked for larvae after 4-5 days. Paddles were collected separately, put in to a basin with aerated tap water and kept for another 5-7 days, allowing adequate time for the eggs to hatch. All larvae immerged were counted and identified at their 3^{rd} / 4^{th} instar.

Rainfall measurements for each month were made using a rain gauge and gathered results were confirmed by the Department of Meteorology, Colombo. The number of DF and DHF cases reported in Maharagama, Boralesgamuwa and Piliyandala areas were obtained from the Epidemiological Unit, Department of Health, Colombo. Data collected were used to calculate OI, the percent number of *Aedes* positive ovitraps to the total number of recovered ovitraps. These data were used to determine the relationship of the rainfall and the *Aedes* mosquito density.

Results

Out of 216 potential breeding sites inspected, 24 places were found positive for mosquito larvae. Only one breeding place was detected positive for *Ae.aegypti* while 14 habitats were confirmed positive for immature stages of *Ae.albopictus*. Species of *Armigeres* and *Culex* were also observed. The most abundant mosquito species found within the university premises was *Ae. albopictus*, the secondary vector of Dengue virus.(Figure.2)

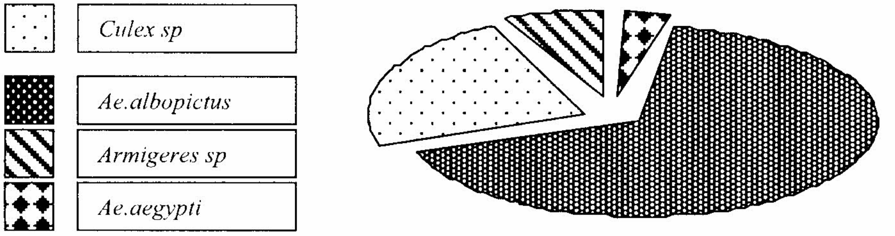


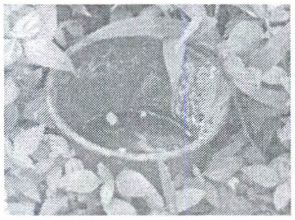
Figure 2. The relative abundance of mosquito species found within the University premises.

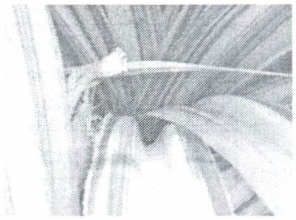
The Container Indices (the percent number of positive containers to the total number of containers observed) for each mosquito species are shown in Table 1.

Table 1. Container Index values for mosquito species found in the University premises during the month of May 2008.

Mosquito Species	**Total number of containers observed**	**number of containers positive for each species**	**Container Index value**
Aedes aegypti	216	1	0.46
Aedes albopictus	216	29	13.43
Culex sp	216	10	4.63
Armigeres sp	216	3	1.39

The highest contribution of 17.26 % breeding places was given by ornamental plants (*Bromellia sp)* while 10.34% formed by discarded plastic buckets,Youghurt cups and opening ends of buried PVC pipes, discarded polythene and plastics formed 6.8%, Bifurcation of trees contributed 5.41% and blocked drainage cannels 5.54%. Some of the major breeding habitats are shown in figure 3.

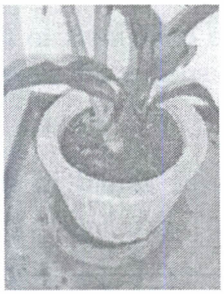
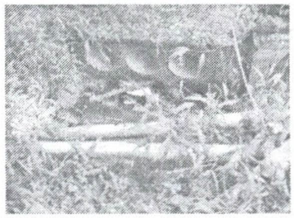
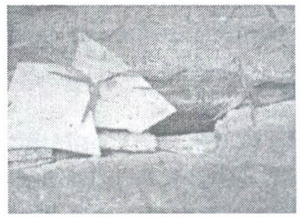




Water filled plastic bucket.

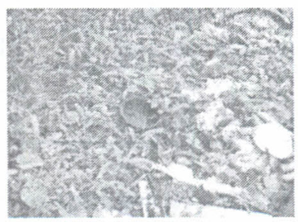
Leaf bases of *Bromellia sp*

Leaf bases of *Pandanus* sp

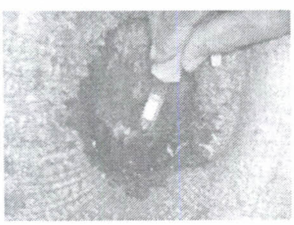
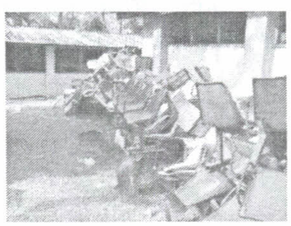




Water filled cement plate under Flower plots

Water collecting concrete pits not properly covered

Discarded garbage with water accumulating pots


Bifurcation of trees

Discarded plastic garbage

Discarded metal

Blocked locations of drainage canals

Artificial bird baths

Figure 3. Breeding places of *Ae.albopictus* mosquitoes within the University premises of Sri Jayewardenepura.

The potential mosquito breeding habitats and those breeding of *Ae.albopictus* are indicated on GPS map shown in Figure 4.

Breeding site positive for ***Aedes aegypti* / *Aedes albopictus***

Possible breeding Site for mosquitoes

NOTE: Breeding sites possible for *Aedes aegypti /Aedes albopictus* –Sites1,4,6,8,7, 14,15,16, 18,20,28,30,28

Breeding sites possible for all mosquitoes –Sites 2, 3, 9, 11, 12, 21, 22, 44, 38, 42, 43, 41, 49, 24, 25, 26,27, 23, 50, 46,19,47,50,51,39,40

Figure 4. Possible mosquito breeding places and the positive breeding habitats of *Ae.albopictus*

The results of the Ovitrap survey is shown in table 2.

Table 2. Average Monthly Ovitrap Index (OI) (%) in the study area.

Month	**OI for *Ae.albopictus***	
	Indoor	**Outdoor**
June	0	**0**
July	0	**2.5**
August	0	**12.5**
September	13.3	**26.0**
October	26.2	**63.1**
November	**39.6**	**80.0**

A total of 180 ovitrap were used for the survey. The OI for *Ae.aegypti* could not be calculated as all ovitraps placed were negative for this mosquito species.

Indoor ovitraps during the period October to December, 2008 and those placed outdoor during June to December 2008 were positive for *Ae.albopictus* giving a Mean Monthly OI of 13.3 and 35.3 respectively.

The breeding of these mosquitoes in outdoor containers was significantly higher than the indoor breeding ($P<0.05$).

During the period where the rainfall was reported low (monthly range-45.6mm-152.6mm), a low range for OI was recorded. (Out door OI = 0-12.5, Indoor OI=0). However, there was an exponential increase in the OI (Indoor = 13.3-39.6, Outdoor=29.6-80.0) within the high rainfall period, September to November (Monthly range- 294.9mm-414.4mm).In the month of September *Ae.albopictus* were recorded in indoor ovitraps. (Figure 5)

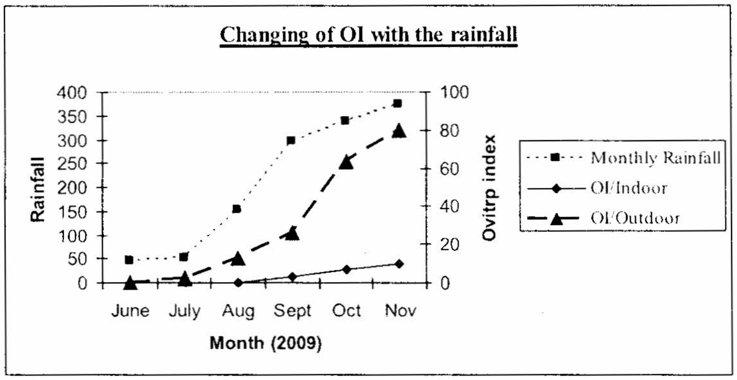


Figure 5. Changing of the OI with the rainfall

Monthly DF cases reported from neighboring areas (Maharagama ,Piliyandala and Boralesgamuwa) increased with the rainfall from September to November 2008 (Figure 6).

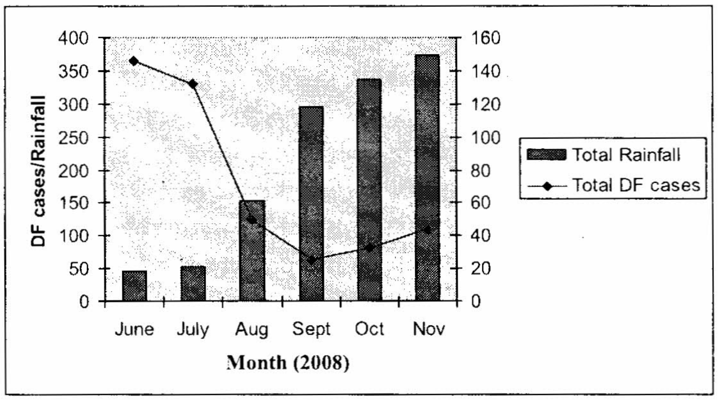


Figure 6. Monthly DF cases recorded from Maharagama ,Piliyandala and Boralesgamuwa areas and the Mean Monthly Rainfall from September to November 2008.

Discussion

Ae.albopictus, an opportunistic breeder, is found commonly in outdoor breeding habitats, including leaf bases of most of the water collecting plants.

According to previous studies on the association between rainfall and larval indices, the main breeding sites were found to be rain exposed cans, discarded bottles, plastic food containers and coconut shells (Gottel,*et al*,1980, Winch, *et al*, 1992). In this study too, the population of *Ae.albopictus* did follow a positive association with the monthly rainfall, with a marked seasonal fluctuation ($P<0.05$), probably because it prefers natural outdoor containers. The marked reduction of outdoor OI in the month of September may be due to wash off of eggs with the onset of high rainfall which occurred after the dry period (May to August).

It was reported in previous studies that *Ae.albopictus* was the predominant species in areas where DF and DHF are endemic (Hapugoda, *et al*, 2004). In Sri Lanka, *Ae.albopictus* is reported to be capable of transmitting all 4 serotypes of the dengue virus. The detection of the dengue virus serotype 3 from *Ae.albopictus* indicates the important role this species possibly play in transmitting the dengue virus in some endemic areas in the country (Hapugoda, *et al*.,2004). The high values of CI and OI for *Ae.albopictus* mosquitoes within the study area reflect that the population of the University is at a risk of acquiring the virus as the transmission of DF and DHF depends on the presence of the virus, *Aedes* mosquitoes and a susceptible population.

The *Aedes* populations could be monitored continuously throughout the study period using ovitraps. The number of DF cases was high in the month of June to July despite the low amount of rainfall obtained. During this period, the breeding of *Ae.albopictus* was low with the OI of 0 to 12.5, but the virus can be spread to the host by *Ae.albopictus* .With the onset of the rainy season in the month of September, the breeding of *Ae.albopictus* was increased (Table 2). The data gathered from ovitraps would serve as a kind of early warning system and assist decision makers to implement suitable activities in vector control to prevent a major outbreak of dengue. Further, the similarity of the climate, vegetation and the geography of the study area with the neighboring dengue endemic areas indicate the possibility of using the high OI data to implement control activities to minimize DF cases in these areas.

Precise locating of mosquito breeding sites using GPS points is important in surveying large areas. Such facilitates to locate true breeding places for mosquitoes would be useful to implement control measures.

While a vaccine is under research without immediate prospect of success, vector control remains the only way to prevent dengue transmission (Guzman *et al*,2002 and Bandaranayaka, *et al*, 2009,). Vector control programmes are essentially based on source reduction, eliminating larval habitats of *Aedes* mosquito from domestic environment (Guzman et al., 2003). The present study was carried out while chemical control of mosquitoes by spraying and fogging, continuous clearing process by the University cleaning service were underway. The results of this study shows the inappropriateness of source reduction alone as a control method when it is launched in an area where the

Ae.albopictus population is drastically high. This is further emphasized as *Ae.albopictus* breeds in a wide variety of natural containers. Similarly, removing water storing plants (whether they are ornamental or not) may lead to social and environmental problems. The inability of applying source reduction alone as a control method is further complicated with the ability of *Ae.albopictus* mosquitoes to breed in places which are practically inconvenient to reach. Roof gutters provide very good breeding habitats when they are blocked with dried leaves. Though a routing cleaning could be recommended, it would be impractical due to lack of adequate manpower and the inconvenience in reaching these sites. Therefore, any other applicable control measures would be needed for effective control of *Aedes* breeding spraying application of Bti (*Bacillus thuringiensis isralensis*).

Since the month of June is the peak of dengue epidemic in Sri Lanka implementing control measures from May to September would be effective to control an epidemic out break. Integrated Vector Control (IVC) method is suitable to control such an epidemic where chemical control, biological control, physical control (Source reduction) methods are carried out simultaneously based on the nature of breeding habitats. WHO (World health Organization) recommended chemicals with the correct dosage should be used in chemical control engaging trained workers. Releasing *Bascillus thuringiensis isralensis* (Bti) bacteria to breeding habitats that are difficult to reach, releasing larvivourus fish (*Poecillia reticula,Rasbora carvari*) and larvae of predatory mosquitoes (*Toxorhynchites* larvae) are some of the biological control methods that can be recommended to areas with DF cases and *Ae.albopictus* predominantly present. Flushing and continues cleaning of drainage systems would be conducive to prevent breeding of *Aedes* mosquitoes in close premises of man. It would be more effective to launch source reduction campaigns before the onset of the rainy season while some control methods such as releasing of mated *Toxorhynchites* mosquitoes could be implemented during the rainy season.

Though the dengue virus had been isolated from wild caught of *Ae.albopictus* mosquitoes (Hapugoda et.al. 2004), its role as a potential vector of dengue is yet to be identified. The emergence of a new serotype of Dengue virus differing at one or more critical neutralization epitopes will undoubtedly occur at some time in the future (Monath, 1994).It will make the situation of a Dengue epidemics more complicated in the country. Therefore research studies should be continued to study the role of *Ae.albopictus* as a reservoir of dengue virus in dengue endemic regions in Sri Lanka.

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