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POPULATION DYNAMICS AND BREEDING PREFERENCE OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* ATTRIBUTING TO DENGUE THREAT IN KELANIYA MEDICAL OFFICER OF HEALTH AREA IN SRI LANKA

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ABSTRACT

Aedes aegypti and *Ae. albopictus* are the two species of dengue vector mosquitoes in Sri Lanka. Indoor and outdoor distribution of these species, their population dynamics and breeding preference are important factors determining the dengue transmission. Gampaha district in Sri Lanka reported to have frequent dengue epidemics from recent past, hence this study was conducted. Ovitrap surveillance was conducted to determine the vector species distribution in indoor and outdoor in Kelaniya Medical Officer of Health (MOH) area of the Gampaha district. Larvae were reared until emergence of adults in the laboratory to identify species morphologically. Breeding preference of vector mosquitoes were carried out by physically observing the presence of mosquito larvae in water accumulated receptacles in the area. *Aedes aegypti* and *Ae. albopictus* were reported to distribute throughout Kelaniya MOH area as a mixed population. *Ae. albopictus* is the most abundant species both indoor and outdoor in Kelaniya MOH area compared to *Ae. aegypti*. Weather factors, rain days and wind positively correlated with *Aedes* population. High risk areas were identified as Kiribathgoda and Hunupitiya in this study.

Key words: breeding, oviposition, ovitrap, surveillance

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INTRODUCTION

Among the *Aedes* species mosquitoes, *Ae.* (sub genus s*tegomyia*) *aegypti* (Linnaeus, 1762) which is a predominantly urban species, and *Ae.* (sub genus s*tegomyia*) *albopictus* (Skuse, 1894) which is a peridomestic species, are the two species of dengue vectors in Sri Lanka (Vitharana et al. 1997; Sirisena & Noordeen, 2014). Both these species are prevalent throughout the country in both urban and semi urban areas (Sirisena & Noordeen, 2014). They only need clear water, but not necessarily clean water to complete the life cycle (Lee, 1991). They prefer to breed in low values of turbidity and BOD (Biological Oxygen Demand) which are critical parameters of mosquito breeding water (Dom et al. 2016). Breeding habitats of these species include variety of water-containing receptacles such as discarded tires, flowerpots, drums, plastic buckets of various sizes, polythene bags, dishpans, discarded plastic drinking cups, crushed aluminum beverage cans and cemetery vases as well as natural breeding sites such as flooding areas, coconut shells and king coconut husks, leaf axils and tree holes (Nelson, 1986; Winch et al. 1992; Garcia et al. 2011). *Aedes* eggs can withstand desiccation, remain dry for months but remain viable. Such eggs hatch when soaked in water (Nelson, 1986; Service, 2012). *Aedes* larval habitats include both outdoor *i.e.* outside of residential houses and indoor *i.e.* inside of residential houses. Depending on the temperature and the availability of food, *Aedes* larvae can complete their larval development between five and ten days (Lee, 1991).

Aedes mosquitoes are very aggressive daytime biters with peak biting periods generally occurring during the early morning and late afternoon. They feed on a large number of hosts including man, domestic and wild animals and this generalized feeding behaviour contributes to its vector potential. A female mosquito has to obtain blood meal for eggs development. Habitats of the females can be permanent stagnant water, flowing water, temporary stagnant water or containers. *Aedes albopictus* is an opportunistic container breeder that can utilize natural as well as artificial container habitats for their breeding. It can adapt to an exceptionally wide range of confined water sources. The mosquito is known for its ability to survive in very small collections of water, requiring only a depth of 0.5 cm to complete its life cycle**.**

Temperature, rainfall, wind speed and direction and its relative humidity also influence the abundance of the mosquitoes. However, in each country where there are enough susceptible hosts, these factors need to be associated with suitable climatic conditions before dengue fever can establish, since it is transmitted by mosquito species whose life cycle is influenced by temperature, humidity and rainfall (Morin, et al, 2013).

In tropical and subtropical climates, *Aedes* species is abundant all year round. Clearly changed seasonal pattern does not show with weather in Sri Lanka. Therefore, a significant difference in larval numbers cannot be expected over the year. However, the changes of climatic factors and geographic factors provide a suitable condition for *Aedes* breeding and distribution. Rainfall is the most important factor that affects *Aedes* breeding. Mosquitoes are also sensitive to temperature changes as immature stages and as adults. If the water temperature rises, the larvae take shorter time to mature (Rueda et al, 1990) and consequently there is a greater capacity to produce more offspring during an epidemic. Adult female mosquitoes digest blood faster and feed more frequently in warmer climates, thus increasing transmission intensity (Gillies, 1953). However, warming above 34 °C generally has a negative impact on the survival of vectors and pathogens (Rueda et al, 1990). High relative humidity can give high hatching rates. With the tropical weather in this country, the high relative humidity has little impact on eggs development as reported by Manorenjitha, 2005. According to the source of epidemiological unit in Sri Lanka Gampaha was the secondly highest dengue threat spot next to Colombo district. Kelaniya MOH area of the Gampaha district, is reported as an area with frequent dengue outbreaks. The objective of this study was to study the population dynamics of two dengue vector mosquito species, *Ae. aegypti* and *Ae. albopictus* and their breeding preferences in Kelaniya area in Gamapaha District in Sri Lanka.

MATERIALS AND METHODS

Study area: Kelaniya Medical Office of Health (MOH) area in Gampaha district, Sri Lanka comes under administrative regions of Peliyagoda Urban Council and Kelaniya regional council. This includes 37 divisional secretariats and six Physical Health Inspecting (PHI) areas namely Kelaniya, Kiribathgoda, Peliyagoda, Wedamulla, Dalugama and Hunupitiya Figure 1. Kelaniya MOH area lies between 6.9687'47''- 6.9983'5'' latitude and 79.056'11''- 79.934'54'' east longitude. Altitude is about 23.0 metres above mean sea level. The area has about 132,655 human population distributed in 2366 hectare (Department of Statistics, Sri

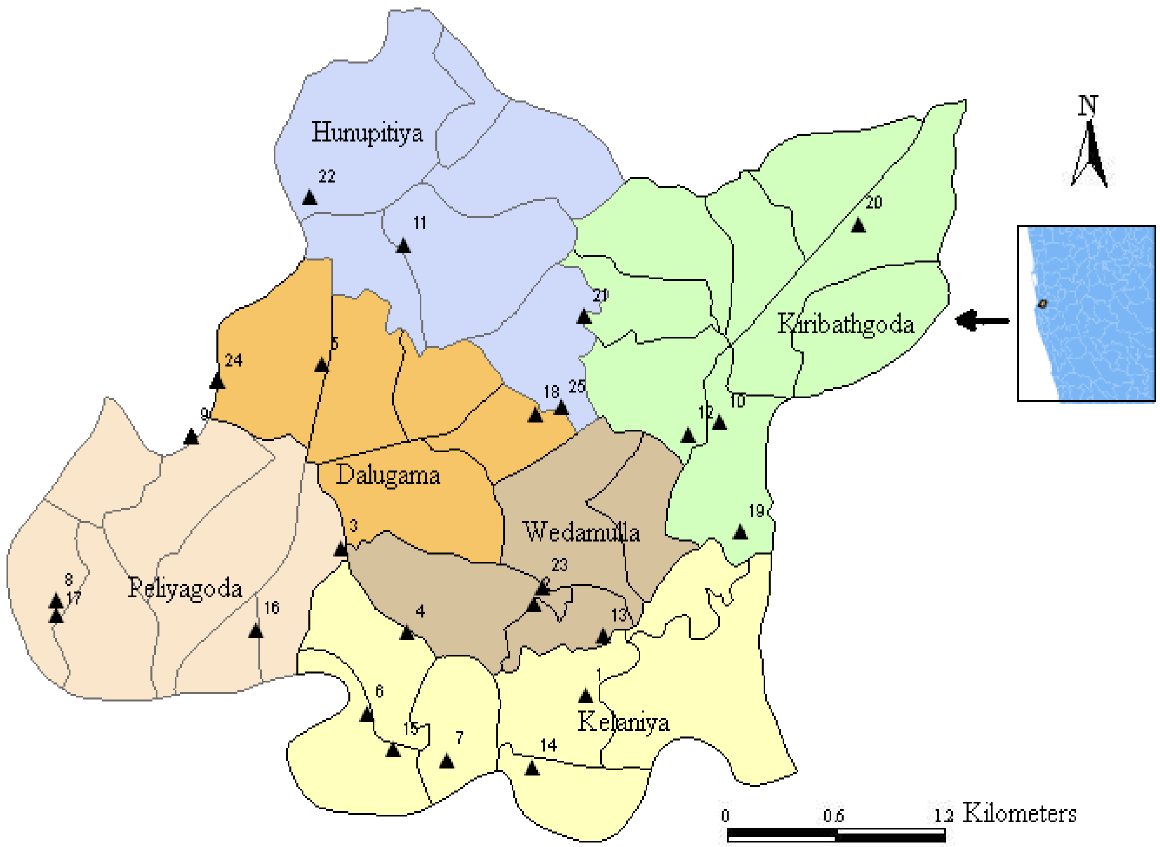

Figure 1: Map of Kelaniya MOH area showing the six PHI divisions, 37 Divisions of secretariats and the sites selected for the ovitrap installation (▲1-25).

Lanka, 2002). Climate is characterized as slightly dry with a range of 27 - 30 ºC temperature. The average annual rainfall varies from 1000 – 1578 mm occurring mainly in the month of May. Relative humidity ranges from 70% - 80%.

Descriptions of study locations under each PHI area describes as follows.

Hunupitiya

It is a sub urban area; lays through 407 ha with 22,370 human population. There are abandoned and dense gestation with vegetation cover (Plate 1 a, b, c). Marshy lands occupy with *Eichhornia crassipes* (Water hyacinth) and *Annona glabra* (Pond Apple). There is no proper drainage system and disposal management. Residential houses are built in refilled wet-lands with no facility for proper drainage.



Plate 1 a,b,c

Kiribathgoda

This division is the smallest division with 11,178 human population in 246 ha in Kelaniya MOH area. It is an urban area which is also with wet-lands that undergo flooding throughout the year. Filled wet-lands cause for poor drainage system and disposal management (Plate 2 a, b).


Plate 2 a,b

Dalugama

This is a sub urban area with 30,820 human population. Total 471 ha area which is also with wetlands undergoing flooding in most of the time of the year. It is with poor drainage system and poor disposal management (Plate 3).

Plate 3

Wedamulla

This division has the 30,337 human population in 332 ha, with the highest population density of 91 people. It is a rural area with proper drainage system and disposal management (Plate 4).

Plate 4

Peliyagoda

It is an urban area with 25,854 human population in 424 ha area with poor drainage system and poor disposal management (Plate 5 a, b). There are marshy lands and wet lands and shanties.


Plate 5 a, b

Kelaniya

It is the largest PHI division with 12,096 human population in 486 ha with lowest population density of 25 people per ha. It has a poor drainage system (Plate 6 a, b). Area covers with marshlands.


Plate 6 a,b

Dengue vector mosquito ovitrap surveillance

Ovitraps were set up at randomly selected twenty-five households covering all PHI divisions, after a verbal consent obtained from the residents. Ovitraps were made using 250 mL plastic containers (10 cm diameter) which had been painted with black enamel paint (Figure: 2). Containers were placed at the rate of three indoor and three outdoor at each residence in the morning each month commencing from January 2009 to December 2009. Space between two indoor ovitraps was decided based on the size of the house whereas distance between two outdoor ovitraps was minimum of one meter radius. A volume of 175 mL of the hay infusion which was made using 126 g of hay soaked in 15 litre of water for seven days and fermented for seven days was poured into each cup and a flat wooden stick (10 cm long and 1 cm wide) was angled inside the cup to facilitate the mosquito oviposition. Wooden sticks were removed from the container after two over nights and brought to the laboratory in rigiform cases. Wooden sticks were held individually under stereo microscope to observe *Aedes* eggs. The number of eggs per ovitrap of indoor and outdoor was calculated and tabulated for each study site. Mean No. of eggs per *Aedes* mosquitoes per ovitrap were designated as low (L) 0-10 eggs, moderate (M) 10-20 eggs and high (H) >20 eggs.

Figure 2: Ovitrap made up of 250 mL plastic container filled with hay infusion

After the egg count wooden sticks bearing mosquito eggs were placed separately in cylindrical transparent plastic containers (7 cm height x 11 cm diameter) partially filled with hay infusion. Each container was covered using a mosquito proof net. After the eggs hatch developing larvae were fed with fine powdered fish meal until pupation laboratory conditions (temperature of 25 ± 3°C; relative humidity 74-86%). Emerged mosquitoes were anesthetized using ethyl acetate and were identified using WHO guidelines (WHO, 1995).

Dengue vector mosquito larval surveillance

All types of water holding containers, coconut shells, water retaining trees/plant parts, roof gutters, indoor ponds and open concrete slabs that are of potential dengue vector mosquito breeding habitats at each household premises and home gardens until totaling to 100 were sampled for mosquito larvae and pupae once for the study period. Mosquito larvae and pupae were collected from each, into a wide mouth transparent plastic bottle by pipetting or using a standard metal scooper depending on the volume of the water collection. Samples were labelled and brought into the laboratory. Identification of the *Aedes* larvae was done to the species level (WHO, 1995).

Dengue risk mapping in Kelaniya MOH area

Geographic conditions such as vegetation cover, land use pattern, Locations of water bodies and mosquito breeding areas including man-made containers, vegetation and other water bodies also were recorded and mapped using Global Positioning Systems (GPS). Boundary of the study area was digitized using MapInfo software or Arc View and available large scale paper maps and digital maps. Population data were obtained from Census Department. Date on exact locations of objects such as houses, waterbodies, roads, streams which determine the risk areas of dengue were collected using GPS technology. These digital data were stored in separate tables using GIS. Data on different types of land use pattern and the way the land were utilized were analyzed using the GIS facility. A database was created in geographical information system for dengue risk categories and linked with spatial point data of each object. The GPS locations of patients were converted into a shape file. The base map of the study area (scale 1: 10,000) purchased from the Survey Department of Sri Lanka was also brought into ArcMap 9.2. Finally, all maps were overlaid to generate dengue risk map.

Meteorological data

Meteorological data such as monthly temperature, humidity, rainfall, number of wet days, and wind direction relevant to the closest vicinity to study area were taken from the Meteorology Department.

RESULTS

Results obtained for the ovitrap survaillance over a period of one year showed that Hunupitiya, Kiribathgoda, Peliyagoda and Kelaniya divisions recorded a presence of higher *Aedes* mosquito populations compared to other two divisions (Figure 3). It was evident that a moderate number of eggs (10 – 20) were recorded from Dalugama division and the lowest was from Wedamulla division. Significantly higher egg counts were recorded in outdoor traps than in indoor traps except in Dalugama PHI division in which egg counts in indoor and outdoor ovitraps were not significantly different. Graphical presentation of mean number of *Aedes* eggs recorded in outdoor ovitraps in each month over the year shows that there are two prominent peaks each in April and July (Figure 4). However, it shows a slight increase of indoor egg count in April.

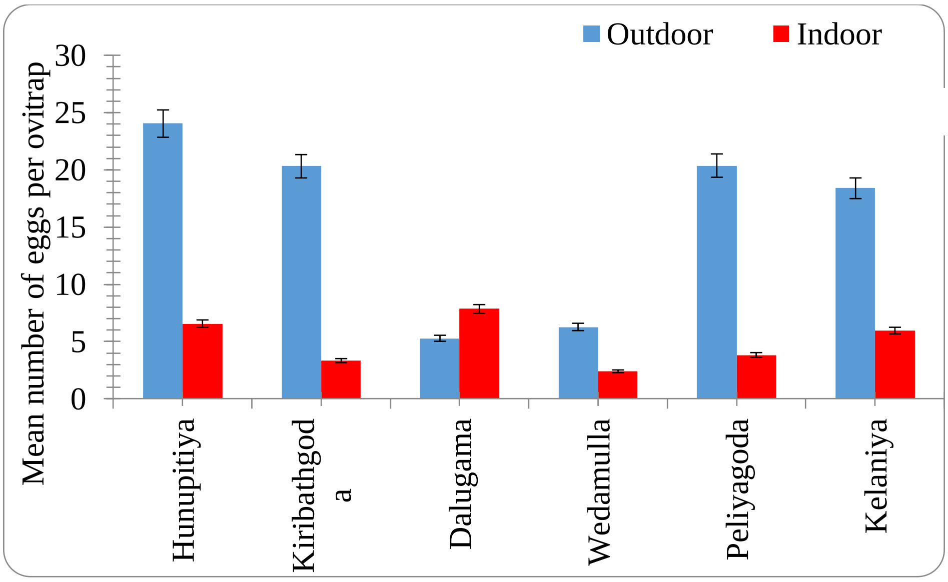

Figure 3: Mean number (± SE) of *Aedes* eggs encountered per ovitrap in outdoor and indoor locations in each PHI division

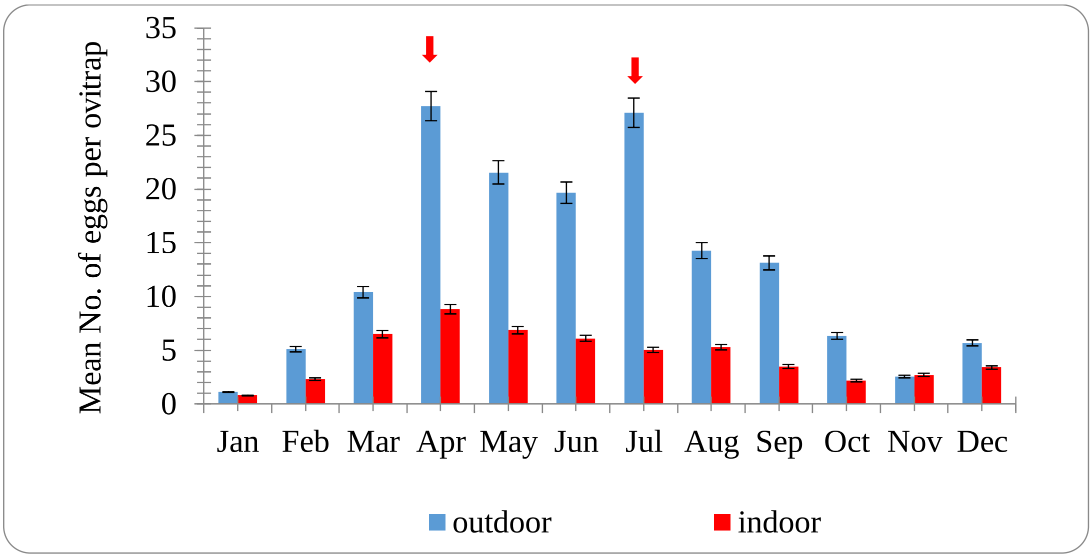

Figure 4: Mean number (±SE) of *Aedes* eggs encountered per ovitrap in outdoor and indoor locations over the year

Aedes adult mosquitoes emerged from eggs collected from ovitraps were resulted both *Ae. albopictus* and *Ae. aegypti* in varying compositions. Very occasionally, *Aedes vittatus* was recorded. *Aedes* mosquito species abundance in indoor and outdoor revealed that the dominant species was *Ae. albopictus* (79%) both in indoor and outdoor (Figure 5).

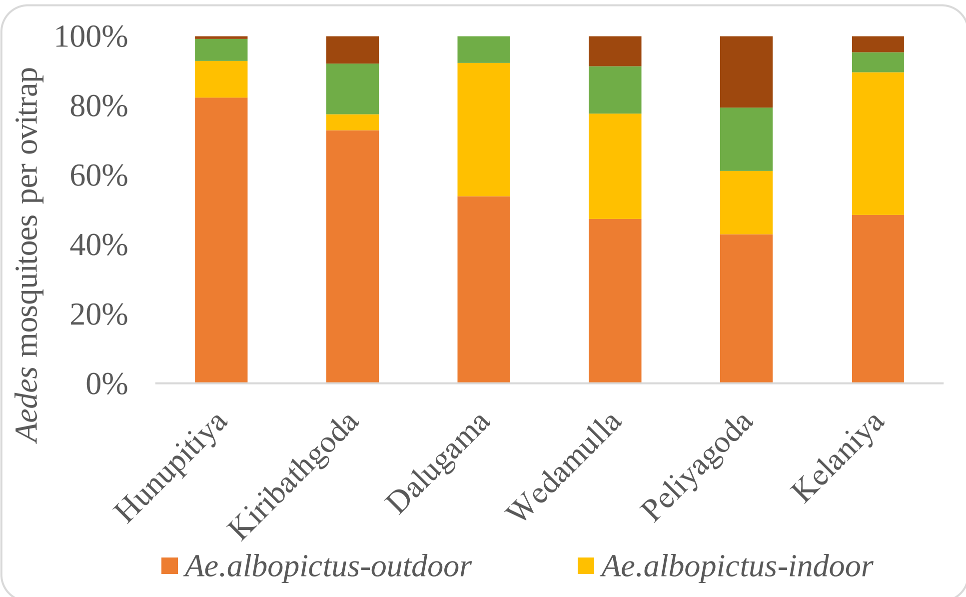

Figure 5: Occurrence of *Ae. albopictus* and *Ae. aegyptii* based on ovitrap surveillance in indoor and outdoor in each PHI division

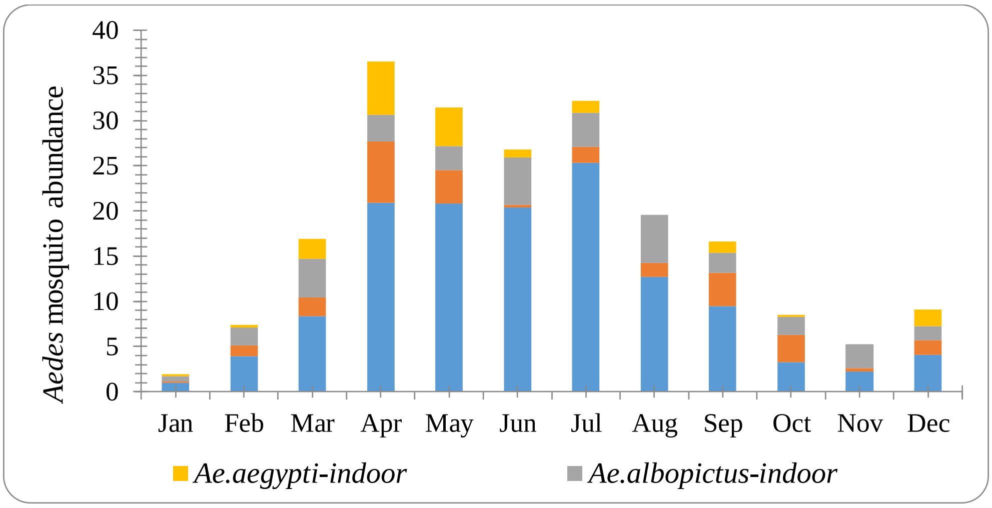

Figure 6: Temporal variation of abundance of *Ae. albopictus* and *Ae. aegypti* per ovitrap

There is a significant positive correlation between outdoor and indoor population of *Ae. aegypti* (R^2 = 0.823, P = 0.044). There is a spatial difference of *Ae. aegypti* both in indoor and outdoor (R^2 = 91.67%, P=0.012). Graphical presentation based on the *Aedes* mosquitoes emerged from ovitrap collection over the year shows that there are two peak population each in April and July.

Mean number of *Ae. albopictus* recorded per ovitrap in both indoor and outdoor in each month shows that there is a higher mosquito population during the period from April to July (Figure 6). In contrast, a lower count of *Ae. aegypti* was recorded throughout the year except in outdoor count in April.

Mean number of *Aedes* eggs recorded per ovitrap in outdoor was positively correlated with respective monthly mean rainfall (Figure 7; p = 0.028, r^2= 0.630) and number of rainy days per month (Figure 8; p = 0.012, r^2 = 0.697).

Mean number of eggs recorded in outdoor ovitraps was significantly correlated with monthly mean humidity (p = 0.038; r^2 = 0.603) and humidity in daytime (p = 0.004; r^2 = 0.764). Mean number of eggs in outdoor ovitraps was positively correlated with monthly mean value of minimum temperature (p = 0.005; r^2 = 0.755) and negatively correlated with monthly highest temperature ($p = 0.007$; $r^2 = - 0.732$). However, there was no any significant relationship between mean number of *Aedes* eggs and monthly mean wind speed.

Mean number of eggs recorded in indoor ovitraps was significantly correlated with respective monthly mean rainfall ($p = 0.000$; $r^2 = 0.854$); humidity in daytime ($p = 0.046$; $r^2 = 0.584$) and monthly mean value of minimum temperature ($p = 0.037$; $r^2 = 0.605$). However, there was no any significant relationship between mean number of *Aedes* eggs in indoor ovitrap and monthly mean wind speed.

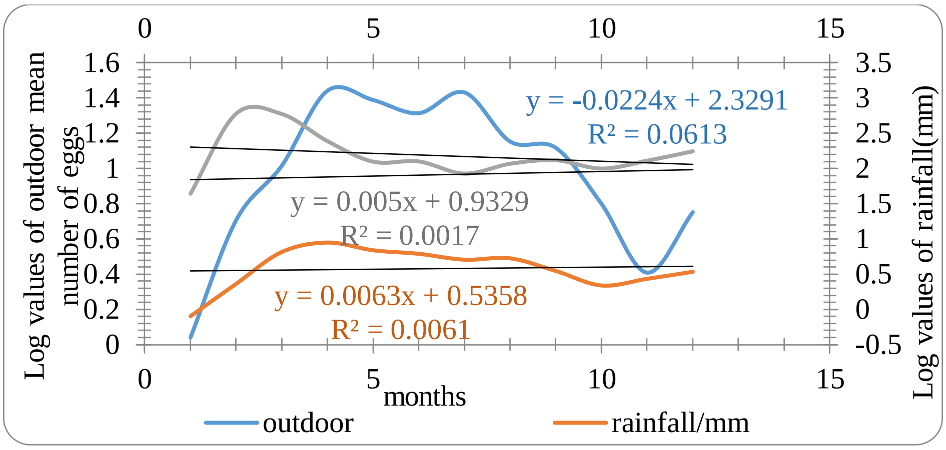

Figure 7: Correlation between mean number of eggs in outdoor per ovitrap and respective monthly mean rainfall and rainfall per rainy day

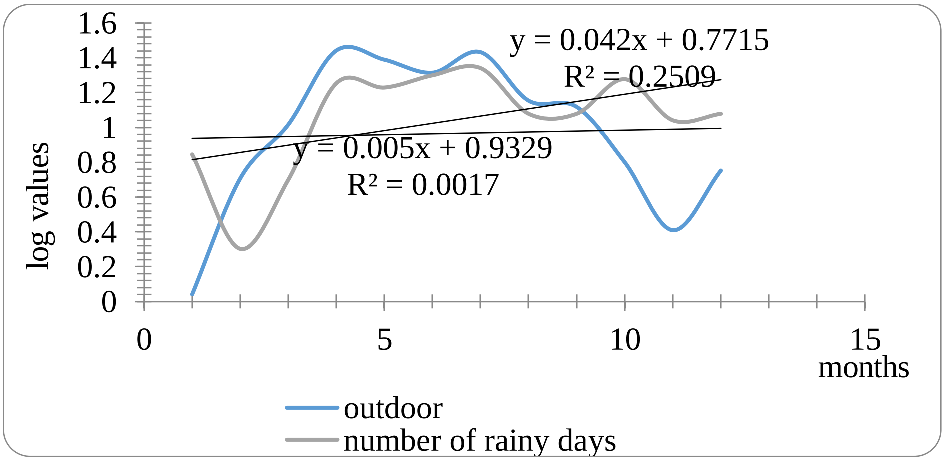

Figure 8: Correlation between mean number of eggs in outdoor per ovitrap and respective monthly number of rainy days

Dengue vector mosquito larval surveillance

Discarded tires, containers, coconut shells, and water retaining tree holes/ leaf axils, roof gutters, indoor-ponds and open concrete slabs were reported as potential breeding sites of dengue vector mosquito species in house premises. The frequently found breeding habitats in house premises of all the PHI divisions are the water filled discarded containers (Table 1). Water retaining tree holes/leaf axils are also significantly contributed to the mosquito breeding in house premises of all the PHI divisions.

Dengue risk mapping

Dengue risk map was created considering the *Aedes* mosquito population based on ovitrap and larvae surveillance (Figure 9). Kiribathgoda and Hunupitiya areas were identified as high risk areas in this study.

Table 1: Frequency occurrence of *Aedes* mosquito breeding habitats in PHI divisions

	Hunupitiya	Kiribathgod	Dalugama	Peliyagoda	Wedamulla	Kelaniya
Tires	9	2	3	4	5	4
Coconut shells	3	8	6	12	8	13
Containers	59	54	55	43	47	53
Domestic ponds	2	3	3	3	3	5
Concrete slabs	7	10	6	11	9	0
Construction buildings	2	2	1	8	5	4
Roof gutters	8	10	10	7	12	15
Tree holes/ leaf axils	10	11	16	12	11	6
Total	100	100	100	100	100	100

DISCUSSION

The main risk factor of the Dengue and dengue haemorrhagic fever in Sri Lanka is the prevalence of vector mosquito species, *Aedes aegypti* and *Ae. albopictus*. Vector population enhances by indoor and outdoor water accumulated receptacles facilitating mosquito breeding. Therefore, it is warranted to understand the breeding preference of the dengue vector mosquitoes and the population dynamics to prevent dengue outbreak in high-risk areas.

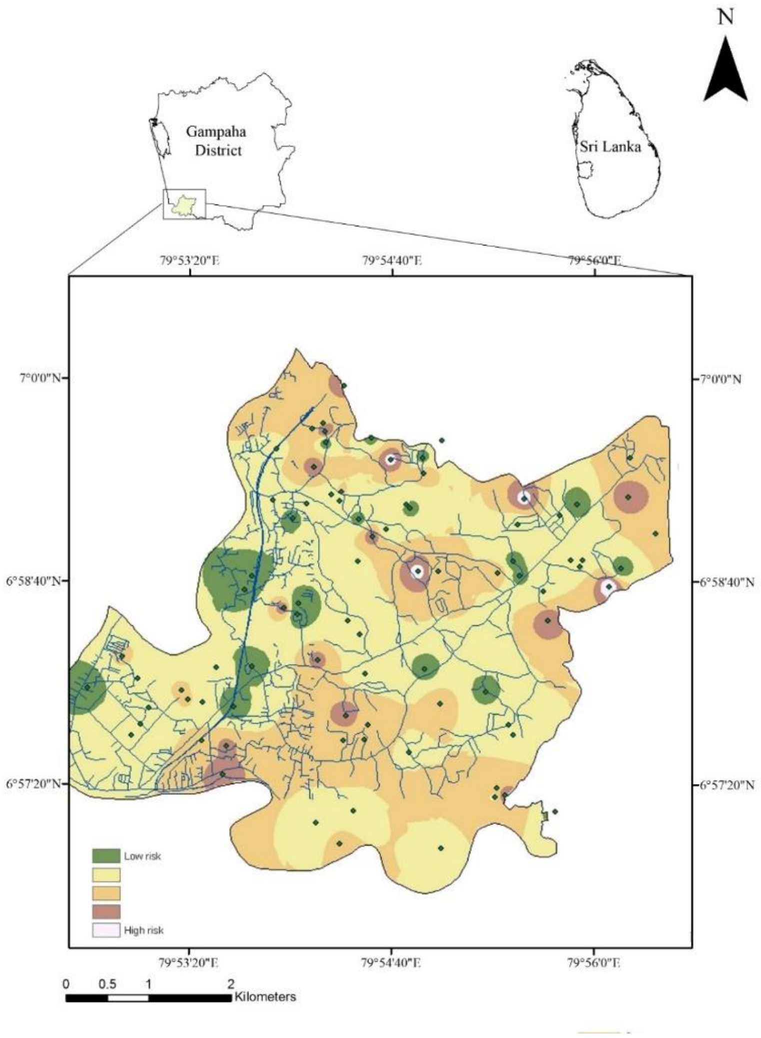

Figure 9: Dengue hotspots in Kelaniya MOH area. Black colour dot marks – larvae surveillance points. Blue lines are the main roads.

Weather factors such as monthly rainfall, number of rainy days, rainfall per wet days, temperature and humidity were significantly correlated with the monthly mean number of *Aedes* eggs and dengue vector mosquito species abundance. Ovitrap collection from March to July gave a peak in April. This was positively correlated with monthly total rainfall, number of rainy days, rainfall per wet day and wind speed. Breeding habitats of *Aedes* mosquitoes were rapidly created with water collecting receptacles in rainy season. Further, the range of monthly temperature between maximum and minimum values and the range of monthly humidity between day and night values were low during this period.

There is a slightly lower abundance of both species of mosquitoes during the dry season compared to the rainy season (Pinheiro & Tadei, 2002). Mosquito larval development is highly dependent on temperature. High moisture content helps to break resistivity of mosquito eggs and induce egg laying of female.

Ovitrap indices based on ovitrap surveillance within six PHI divisions indicated that Hunupitiya was recorded as higher values whereas Kelaniya and Peliyagoda followed it. This study observed that *Aedes* vectors deposited their eggs in outdoor containers (total eggs = 154/ovitrap) which exposed to sunlight (≥ 40 % of the day) than in indoor containers (total eggs = 57/ovitrap) throughout the study period. Maximum daily water temperatures in each surveyed containers typically 27 °C – 30 °C in outdoor sites and 25 °C – 28 °C in indoor sites. Larval development is highly temperature dependent (Reuda et al, 1990). Female may have a selective advantage if they are able to detect containers with warmer water where their offspring develop faster. Immature *Aedes* vectors were more abundant in shaded containers with low water temperature (≤29°C), indicating that females oviposit more frequently in containers shielded from full sunlight (Barrera et al, 2006). Due to environmental differences between indoor sites and outdoor sites, outdoor containers in Kelaniya MOH area received commonly sun exposure up to 50% of the day, in contrast indoor sites received sun light up to 10% of the day for most indoor containers.

Results revealed that *Ae. albopictus* was higher abundant in both indoor and outdoor. There were many artificial and natural large oviposition sites. During the study period, Hunupitiya and Peliyagoda PHI divisions were under construction sites and there were many artificial containers (65% of containers) and open concrete slab areas (10% of division). Females laid more eggs in cement containers compare to plastic or metal containers when all were similar in size. (Wong et al, 2011). Kelaniya PHI division is bordered from its eastern margin with the Kelani river, hence undergo flooding in heavy rains. There also many abandoned and vacant lands implying to increase ovitrap indices and *Aedes* vector mosquito abundance in Kelaniya PHI division. Rest of other three PHI divisions were recorded with lower ovitrap indices and *Aedes* vector mosquito abundance with the lowest value recorded in Wedamulla.

The wider prevention and control of dengue is currently reliant on vector control methods. These include environmental, biological, and chemical vector control strategies and methodologies. It is therefore important to elaborate on some of the potential factors that drive dengue activity, as well as the global strategic direction to address this growth. Effective vector control is the mainstay of dengue prevention and control.

This study encountered 68.96% of total outdoor breeding sites mainly consist of rainwater accumulated discarded, polythene and plastic (42.65%), coconut shells (33.08%) and tires (7.35%) as suitable receptacles for dengue mosquitoes' oviposition. Among them, discarded used tires with retained rainwater are the main outdoor breeding habitat for *Ae. aegypti* in this study. The present study denotes that 68% of surrounding was sparsely covered with vegetation while another 60% was categorized under built environment. Napier, 2001 stated that the housing pattern and the distance between houses have a significant influence on the dengue outbreak and its distribution trend. Therefore, high human population density and short distance between houses could lead to more efficient transmission of the virus and thus, increased exposure to infection. Simultaneously, the present study encountered that 66% of houses were almost interconnected or located in very close vicinity to each other in a crowded areas with very high human population. However, transmission of the disease is normally limited by the flight distance of *Aedes* mosquitoes in which *Ae. aegypti* could range from a few meters to more than 50 m and less than 500 m in a closed urban environment (Morlan & Hayes, 1958). General expectation is that there is a negative correlation between dengue incidence rate and level of education or the level of awareness of dengue. However, present study revealed that though communities may perform well in terms of knowledge of the disease, they may also do less in attitude and practice against dengue transmission. In the present study, most of the dengue positive cases were found from middle class families and those engaged in any kind of employment. Therefore, it can be presumed that patient number has been increased due to lack of time in spending at home in attending on dengue patient in early stages. This statement was advocated by having only 13% indoor breeding places with reference to outdoor breeding places (87%) supporting to the fact that due the lack of time they may have not concerned on their outdoor surrounding environment that have already provided suitable breeding grounds for the vector mosquitoes. Hence, all these factors have interconnected with dengue situation in this study area.

CONCLUSIONS

Three *Aedes* species were established in the study area with most abundant species was *Ae. albopictus*. *Aedes vittatus* was rare. Dominant two *Aedes* species were found throughout the study period at both sites of indoor and outdoor. Potential highly transmissible period of the dengue disease lie in March to July of the year. Mosquito breeding places have been mainly created due to unplanned urbanization, increasing urban populations, and poor garbage disposal/collection. Rainfall, temperature and humidity are more favourable for increase of the *Aedes* population.

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