USE OF OVITRAP SURVEILLANCE TO ASSESS DENGUE OUTBREAK RISKS IN SELECTED DENGUE ENDEMIC AREAS IN SRI LANKA

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Abstract

Dengue fever has been endemic to Sri Lanka for several decades. Due to the unavailability of an established prophylactic medicine, dengue prevention depends largely on vector control, where vector surveillance plays a key role. The present study aimed to assess the *Aedes* mosquito abundance and the risk of disease outbreak using ovitrap surveillance in 14 areas in Sri Lanka, covering four districts with high dengue incidence during 2014 – 2016. A total of 1537 ovitraps were placed in Colombo (Kirulapone, Dematagoda, Grandpass and Thummulla), Gampaha (Kurana and Imbulgoda), Kalutara (Horana, Keselwaththa and Kalamulla) and Kandy (Nawalapitiya, Peradeniya, Edanduwawa, Hanthana Road and Thalwaththa) districts in both indoor and outdoor sites and were collected after five days. The larval counts were used to calculate the Container Index (CI) and Ovitrap Index (OI). Our results revealed significantly higher CI for *Aedes* species for outdoor compared to indoor sites, indicating a tendency of *Ae. aegypti* and *Ae. albopictus* to breed more in outdoor habitats ($p < 0.05$). In most co-bred ovitraps, the number of *Ae. aegypti* larvae were higher than those of *Ae. albopictus*. The abundance of *Ae. aegypti* was higher in urban areas compared to rural areas ($p < 0.05$) whereas no such difference was observed for *Ae. albopictus*. This suggests that *Ae. albopictus* has been successful in invading habitats with different levels of urbanization. Further, all studied areas showed an OI > 10 % for either or both *Aedes* species reflecting a possible risk of dengue outbreaks as per the guidelines. Nevertheless, only the abundance of *Ae. aegypti* (in terms of OI) showed a positive correlation with the number of dengue cases ($r = 0.96$, $p < 0.05$) indicating its substantial contribution towards dengue incidences in the studied areas.

Keywords: *Aedes aegypti*, *Aedes albopictus*, container index, dengue incidence, larval survey, ovitrap index

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1. Introduction

Dengue is ranked as the most rapidly spreading mosquito borne disease in the world– with a 30-fold increase of the global incident rate over the last 50 years according to the World Health Organization. The disease has affected people in more than 100 countries, mainly in tropical and sub-tropical regions of the world. Each year, 50 to 100 million dengue virus infections along with 20,000 deaths are reported from all over the world (WHO, 2013). The dengue virus is transmitted to humans mainly by two mosquito species; *Aedes aegypti*, also known as the yellow fever mosquito which is the primary vector or the epidemic vector and is abundant in urban residential areas, and *Aedes albopictus* the Asian tiger mosquito, which is the secondary vector or the maintenance vector mostly found in rural and sub urban areas with high vegetation (Lounibos & Kramer, 2016). The world's first dengue vaccine was licensed for use in humans in 2015 (WHO, 2016). However, according to the revised recommendations by the WHO, the use of the vaccine is restricted to those who have been infected with the dengue virus previously. Hence, vector control still acts as the main method of dengue prevention.

Sri Lanka, being a tropical South Asian country, is critically affected by the dengue virus with an increased level of dengue cases and deaths every year. The first serologically confirmed dengue case in the country was reported in 1962 followed by the first island-wide dengue epidemic associated with serotypes 1 and 2, with a total of 51 cases of dengue hemorrhagic fever and 15 deaths during 1965 to 1968 (Vitarana et al., 1997). However, in 2017, 186,101 dengue cases along with 350 deaths were reported island wide which marked the most severe dengue epidemic in the country so far (Epidemiology Unit, 2017). The 23-fold increase in dengue related deaths

together with extremely high numbers of dengue cases over the past half century likely indicate the failure of the prevailing dengue prevention methods stemming from the lack of a sustainable vector control strategy. Further, frequent dengue epidemics imply the failure of the public health system to respond rapidly to the rising numbers of dengue incidences. The timely control of such epidemics requires preparedness and prompt action of the respective authorities (WHO, 2012).

Vector surveillance studies play a key role in ensuring preparedness to tackle any impending dengue epidemic. It assists in detecting vector population dynamics and can be used to monitor and evaluate prevailing vector control activities. Vector surveillance will identify both the geographical areas and time periods where there is a high density of *Aedes* mosquitoes. This information is of great entomological significance, since high population density of the vector reflects increased opportunities of disease transmission (WHO, 2012). Recognizing this fact, the ovitrap was first invented in the United States for surveillance of the principal dengue vector *Ae. aegypti* (Fay & Eliason, 1966; Fay & Perry, 1965) and has been in use ever since as a surveillance tool due to its efficient and cost effective nature in detecting *Aedes* mosquitoes, even at low population densities. Other larval surveys in comparison have failed to produce satisfactory results, for an example when the Breteau Index < 0.05 (Evans & Bevier, 1969; Jakob & Bevier, 1969; WHO, 2003). Further, some previous studies have also shown that ovitrap surveillance is significantly more sensitive in detecting the presence of *Ae. aegypti* compared to the conventional larval surveys through visual inspection (Legall, 1998). Ovitrap are also recommended for areas where *Aedes* mosquitoes have not been established previously such as in international ports of entry i.e. airports and seaports (WHO, 1995) as they enable the early detection of new *Aedes* infestations. Ovitrap are also safe and environmentally friendly

when it is collected before the matured mosquitoes fly away (Chan *et al.*, 1977). At the same time, placing ovitraps will provide the vectors conspicuous breeding habitats for egg laying, limiting them from laying eggs in natural habitats. Thus, if removed on time, the placing ovitraps might be an effective control strategy to control dengue vectors. Many countries have been using modified and improved versions of ovitraps i.e. the autocidal gravid ovitrap (Mackay *et al.*, 2013), adulticidal sticky ovitrap (Ritchie *et al.*, 2003) and lethal ovitrap (Rapley *et al.*, 2009) to control both larval and adult stages of dengue vectors. As such, ovitrap surveillance may serve a valuable tool in implementing dengue prevention strategies in a more timely and effective manner.

The ovitrap method estimates vector abundance based on the Container Index (the percentage of water-holding containers/ovitraps positive for *Aedes* larvae) (WHO, 2003) and the Ovitrap Index (the percentage of positive ovitraps for *Ae. aegypti* or *Ae. albopictus* separately, relative to the number of recovered ovitraps) (Dhang *et al.*, 2005). CI provides information on common *Aedes* abundance in an area while OI infers which dengue vector is dominant in a given area. The latter index is a far more informative parameter than the former in dengue epidemiology as the vectorial capacities of the two-vector species are known to differ from each other. Originally, the Ovitrap Index considered only the egg counts in making estimates, even though more recent studies have developed the concept to include the larval stages that emerge from the hatched eggs (Chen *et al.*, 2006; Cheung & Fok, 2009; Dhang *et al.*, 2005; Gunathilake & De Silva, 2009). As shown by Weeraratne *et al.* (2013), this trend may be supported by the fact that larvae-based vector estimates resemble the actual density of vectors more closely than egg-based estimates, due to the high mortality

rate observed during the transition from egg to larva.

In Sri Lanka, the districts of Colombo, Gampaha, Kalutara, Kurunegala and Kandy are the major dengue outbreak points ranked at the top five positions of the list of dengue incidences every year (Dissanayake, 2018; Epidemiology Unit, 2017; Sirisena & Noordeen, 2014) during the past decade. Previous dengue vector surveillance studies have been conducted in some of these districts and have shown various trends with respect to the abundance and involvement of a particular vector species (Gunathilake & De Silva, 2009; Noordeen *et al.*, 2018; Ramasamy *et al.*, 2011; Weeraratne *et al.*, 2013; Wijegunawardana *et al.*, 2019). For example, Weeraratne *et al.* in 2013 has demonstrated that *Ae. albopictus* predominates over the primary dengue vector *Ae. aegypti* in Kandy and Kurunegala districts. However, long-term validity of such observations could only be certified through regular epidemiological surveillance. On the other hand, no vector surveillance studies have been reported from the Kalutara district demonstrating a lapse in the available epidemiological data on dengue transmission. As such, in the present study, an attempt was made to assess *Aedes* mosquito abundance and the risk of disease outbreak using the ovitrap based larval surveillance in four of the five major dengue outbreak areas in Sri Lanka.

2. Material and Methods

2.1 Ovitrap Surveillance

The study was performed during different time intervals between 2014 – 2016 (Table 1), in fourteen sites in the four districts in Sri Lanka - Colombo (Kirulapone, Dematagoda, Grandpass and Thummulla), Gampaha (Kurana and

Imbulgoda), Kalutara (Horana, Keselwaththa and Kalamulla) and Kandy (Nawalapitiya, Peradeniya, Edanduwwa, Hanthana Road and Thalwaththa), where frequent dengue epidemics had been reported. The study sites were selected to represent different levels of urbanization and accordingly consisted of urban, semi-urban and rural residential areas, coastal fishing areas, low-income houses (shanties), schools and university premises. The ovitraps were locally manufactured as per the specifications by the World Health Organization (WHO, 2003). It comprised of a black plastic container with approximately 250 ml capacity and a height of 12.0 cm. As the breeding medium, approximately 175 ml of 10% hay infusion was used. The hay infusion was prepared by steeping 125 g of dried rice hay in 15 L of dechlorinated water for seven days. As the oviposition surface, a 29.7 cm x 7.0 cm strip of filter paper was laid on the inner wall of the ovitrap, so that half of the paper was kept below the infusion. A total of 1537 ovitraps (Table 1) were placed in both indoor and outdoor sites of randomly chosen houses after obtaining the informed consent of the house owners. The number of ovitraps kept at indoor and outdoor sites varied from one another based on the presence of small children and pets, floor space available and the house owner's discretion. For the purpose of ovitrap placement, the interior of the house under the roof was considered as indoor, while the immediate vicinity of the house was considered outdoor (Dhang et al., 2005). Ovitrap were collected after five days and mosquito larvae and/ or eggs in the collected ovitraps were transferred to the laboratory. Each collected ovitrap was replaced with a fresh ovitrap and this was repeated for 2-5 times at each study site during the surveillance period (Table 1). In the laboratory, the eggs were kept for hatching and larvae were reared up to the 3rd or 4th instar level in plastic containers filled with dechlorinated water that are covered with a net. Fish food was used as the energy source and was put into the containers daily. The grown larvae were

identified using standard taxonomic keys to the species level (Amerasinghe, 1995). The number of ovitraps positive for *Aedes* larvae along with the number of larvae per ovitrap were recorded. Since, all the eggs would not hatch and grow to the adult mosquitoes, only the number of larvae (and not the number of eggs) were counted as it relates more closely to the actual field mosquito populations.

2.2 Data Analysis

Information on rainfall and temperature was obtained from the meteorological stations of the Meteorological Department, Sri Lanka, that were nearest to each of the study sites. The Container Index (CI) or the percentage of the positive ovitraps of the total successfully recovered ovitraps, for either of the two dengue vector species, the Ovitrap Index (OI) or the percentage of positive ovitraps for each species, against the total number of ovitraps recovered successfully, the percentage of mixed breeding, the *Ae. aegypti* to *Ae. albopictus* ratio where mixed breeding was observed and the mean larval count per recovered ovitrap, were estimated for indoor and outdoor locations in all sites. The Container Index and the Ovitrap Index were compared among the study sites in relation to the different urbanization levels using the χ^2 test. The correlation between the calculated parameters and rainfall and temperature were estimated using regression analyses. Since the number of dengue cases were not available for the individual study sites, the Ovitrap Index was averaged over the district and compared with the district-wise dengue incidences (source: Epidemiology Unit, Ministry of Health, Sri Lanka. <http://www.epid.gov.lk/web>).

The statistical calculations were done using SPSS 21 (IBM Corp.) and the result was considered significant if the $p < 0.05$.

Table 1: Details of the sampling sites, total ovitraps placed and recovered in each site and the weather parameters

District	Site	Urban / Rural status	Geo reference	Date of surveillance	Indoor/ Outdoor	Total ovitraps placed	Total ovitraps recovered	Mean temperature (°C)	Mean rainfall (mm)	Number of dengue cases*
Colombo	Kirulapone	Urban residential area	6°52'37.0"N 79°52'53.8"E	Jan – Feb 2015	Indoor	41	39	26.6	65.5	3440
	Dematagoda	An urban school	6°55'24.6"N 79°52'51.2"E	Jan – Feb 2015	Outdoor	68	65	26.6	61	
	Grandpass	Urban slums	6°56'37.0"N 79°52'31.8"E	Jan – Feb 2015	Indoor	115	104	27.0	105.5	
	Thummululla	Urban university premises	6°54'07.1"N 79°51'37.0"E	Jan – Mar 2015	Outdoor	18	12	27.0	86.3	
Gampaha	Kurana	Sub urban coastal and fishing area	7°12'17.0"N 79°50'06.3"E	Oct 2015	Indoor	217	199	27.4	370.6	1709
	Imbulgoda	Rural residential area	7°02'22.0"N 79°59'20.2"E	Aug – Oct 2016	Outdoor	10	6	26.7	61.6	
Kalutara	Horana	Sub urban residential area	6°43'23.2"N 80°04'27.5"E	Aug- Sep 2015	Indoor	56	56	27.7	362	267
	Keselwaththa	Sub urban residential area	6°45'10.5"N 79°54'17.1"E	Sep 2015	Outdoor	62	61	27.4	460.6	
	Kalamulla	Rural residential area	6°34'48.0"N 79°58'48.0"E	Aug – Oct 2015	Indoor	36	36	27.6	231.6	
					Outdoor	189	185			
Kandy	Nawalapitiya	Rural residential	7°02'36.8"N 80°31'09.2"E	Oct 2014	Indoor	57	57	25.1	266.1	289
	Peradeniya	Sub urban university premises	7°15'49.2"N 80°35'46.8"E	Oct 2014	Outdoor	6	6	25.1	436.8	
	Edanduwawa	Rural residential area	7°15'57.3"N 80°34'55.4"E	Oct 2014	Indoor	16	16	25.1	436.8	
					Outdoor	7	7			
	Hanthana Road	Rural residential	7°17'09.1"N 80°37'59.8"E	Oct 2014	Indoor	33	33	25.1	443.3	
	Thalwaththa	Sub urban residential	7°17'30.5"N 80°39'14.4"E	Oct 2014	Outdoor	25	24	25.1	443.3	

*Number of dengue cases for the surveillance period obtained from the Epidemiology Unit, Ministry of Health, Sri Lanka for each district as data for each study site was unavailable Jan: January, Feb: February, Mar: March, Aug: August, Sep: September, Oct: October

3. Results

Of the 1537 ovitraps that was placed at indoor (N=753) and outdoor (N=786) premises in the selected areas, a total of 1442 (93.82 %) from indoors (716) and outdoors (726) were successfully recovered for surveillance (Table 1). The others had been either disturbed/destroyed by human/animal intervention or by natural causes such as wind or rain. Of recovered ovitraps, 502 (34.8 %) were found to be positive for immature insect stages. The majority (97.8 %; 491) of the positive ovitraps were infested with either or both, *Ae. aegypti* and *Ae. albopictus*, while a few were occupied by *Culex* mosquitoes (1.4 %; 7) and other non-mosquito species (0.8 %; 4).

Container Index for *Aedes* species ranged from 6.3 % (Peradeniya) to 61.5 % (Kirulapone) for indoors and from 11.1 % (Thalwaththa) to 73.8 % (Kirulapone) for outdoors indicating a significantly higher prevalence of *Aedes* mosquitoes in outdoor premises ($p < 0.05$) (Table 2). Further, no significant distinction was observed for the Container Indices among the urban, sub urban or rural settings for both indoor and outdoor ovitraps ($p > 0.05$).

The analysis of OI in indoor premises showed that, for *Ae. aegypti*, OI ranged from 0 (Thummululla, Imbulgoda, University of Peradeniya and Edanduwawa) to 51.3 % (Kirulapone) while it ranged from 0 (Kurana) to 25.6 % (Kirulapone) for *Ae. albopictus*. Nevertheless, significantly higher indoor OIs were shown for *Ae. aegypti* in comparison to *Ae. albopictus* in Kurana

Table 2: Container Index, Ovitrap Index, percentage mixed breeding traps, *Ae. aegypti*: *Ae. albopictus* ratio in mixed breeding traps and the mean larval count per ovitrap for *Ae. aegypti* and *Ae. albopictus* for indoor and outdoor locations

Site	Indoor/ Outdoor	Container Index (%)	Ovitrap Index (%)		Mixed breeding (%)	<i>Ae. aegypti</i> : <i>Ae. albopictus</i> ratio in mixed breeding	Mean larval count per ovitrap	
			<i>Ae. aegypti</i>	<i>Ae. albopictus</i>			<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
Kirulapone	Indoor	61.54	51.28	25.64	20.83	2.67:1	2	0.31
	Outdoor	73.85	41.54	46.15	18.75	1.56:1	2.45	3.21
Dematagoda	Indoor	45.19	24.04	23.08	4.26	1.13:1	1.07	0.76
	Outdoor	75	41.67	50	22.22	1.83:1	3.17	2.58
Grandpass	Indoor	21.53	13.39	10.53	11.11	1:1.5	0.49	0.30
	Outdoor	28.57	28.57	0	0	0	2.29	0
Thummulla	Indoor	15.38	0	15.38	0	0	0	0.46
	Outdoor	52.34	29.91	26.17	7.14	1.38:1	0.97	0.80
Kurana	Indoor	26.47	26.47	0	0	0	1.29	0
	Outdoor	37.78	34.44	8.89	14.71	1.63:1	2.27	0.3
Imbulgoda	Indoor	16.07	0	16.07	0	0	0	0.34
	Outdoor	16.39	0	16.39	0	0	0	0.57
Horana	Indoor	36.11	16.67	19.44	0	0	0.67	1.56
	Outdoor	42.70	8.65	37.84	8.86	1:1.06	0.69	3.14
Keselwaththa	Indoor	15.79	15.79	1.75	11.011	1.5:1	0.61	0.04
	Outdoor	16.67	0	16.67	0	0	0	0.17
Kalamulla	Indoor	18.82	2.35	17.65	6.25	3.5:1	0.09	1.02
	Outdoor	37.80	2.44	36.59	3.23	1:1	0.12	1.79
Nawalapitiya	Indoor	11.11	5.56	5.56	0	0	0.11	0.06
	Outdoor	23.81	14.29	14.29	20	4:1	0.29	0.29
Peradeniya	Indoor	6.25	0	6.25	0	0	0	0.13
	Outdoor	31.58	10.53	31.58	33.33	1:4.5	0.11	0.74
Edanduwawa	Indoor	14.29	0	14.29	0	0	0	0.57
	Outdoor	45.45	6.06	42.42	6.67	1:4.3	0.39	2.93
Hanthana Road	Indoor	20.83	8.33	16.67	20	4:1	0.25	0.25
	Outdoor	35	10	25	0	0	0.75	0.65
Thalwaththa	Indoor	27.78	16.67	11.11	0	0	0.56	0.39
	Outdoor	11.11	0	11.11	0	0	0	0.11

and Keselwaththa areas. In Imbulgoda and Kalamulla, *Ae. albopictus* revealed significantly higher OI compared to *Ae. aegypti* in the indoor surveillance ($p < 0.05$). For outdoor premises, OI estimated for *Ae. aegypti* ranged from 0 (Imbulgoda, Keselwaththa and Thalwaththa) to 41.6 % (Kirulapone), while for *Ae. albopictus*, it ranged from 0 (Grandpass) to 46.2 % (Kirulapone) (Table 2). At many outdoor sites, i.e. Imbulgoda, Horana, Kalamulla and Edanduwawa, *Ae. albopictus* was found at a significantly higher abundance in comparison to *Ae. aegypti* ($p < 0.05$). However, in Kurana, a significantly higher OI was observed for *Ae. aegypti* compared to *Ae. albopictus* ($p < 0.05$) for outdoor ovitraps. When the indoor and outdoor surveillance results were combined, the Ovitrap Indices for each individual species showed that it ranged from 0 (Imbulgoda) to 45.2 % (Kirulapone) for *Ae. aegypti* and from 3.2 % (Keselwaththa) to 37.5 % (Kirulapone and Edanduwawa) for *Ae. albopictus* (Table 2).

Among them, the Kurana and Keselwaththa populations showed a significant increase of OI for *Ae. aegypti* compared to *Ae. albopictus* ($p < 0.05$) whereas Imbulgoda, Horana, Kalamulla and Edanduwawa populations indicated significantly high OI for *Ae. albopictus* in comparison to *Ae. aegypti* ($p < 0.05$). However, at Kirulapone, where the highest OIs were observed for both species, there was no significant difference between the abundances of the two species.

The percentage of mixed breeding in all study sites including indoors and outdoors, accounted for 0 – 20.83 % (Kirulapone) and 0 – 33.33 % (Peradeniya), respectively (Table 2). However, there was no mixed breeding found in either indoor or outdoor ovitrap surveillances in Imbulgoda, a rural area where no *Ae. aegypti* was present. In most co-bred ovitraps, the number of *Ae. aegypti* larvae observed were higher than the number of *Ae. albopictus* larvae.

Figure 1: Relationship between mean larval counts (per ovitrap) and Ovitrap Indices for the two species at indoor and outdoor locations (a) *Ae. aegypti* at indoor sites (b) *Ae. aegypti* at outdoor sites (c) *Ae. albopictus* at indoor sites (d) *Ae. albopictus* at outdoor sites (r and p values are from regression analysis).

When OI among those sites with different urbanization levels were compared, *Ae. aegypti* showed a significant increase in urban areas compared to rural areas ($p < 0.05$) whereas *Ae. albopictus* did not reveal any such difference ($p > 0.05$). Further, there was no difference among the level of mixed breeding of the two species in the ovitraps placed in urban, sub urban or rural areas ($p > 0.05$).

For the indoor surveillance, the mean number of larvae per ovitrap was significantly higher for *Ae. aegypti* compared to *Ae. albopictus* in Kirulapone, Kurana and Keselwaththa while it was significantly higher for *Ae. albopictus* compared to *Ae. aegypti* in Thummulla, Imbulgoda, Horana and Kalamulla ($p < 0.05$).

Figure 2: Comparison of the mean ($\pm$ standard error) larval count per ovitrap between and within species for indoor and outdoor locations. Kir : Kirulapone, Dem: Dematagoda, Gra : Grandpass, Thu : Thummulla, Kur : Kurana, Imb : Imbulgoda, Hor : Horana, Kes : Keselwaththa, Kal : Kalamulla, Naw : Nawalapitiya, Per : Peradeniya, Eda : Edanduwawa, Han : Hanthana Road and Tha : Thalwaththa

The mean *Ae. aegypti* larval count observed per recovered ovitrap ranged from 0 (Thummulla, Imbulgoda, Peradeniya and Edanduwawa) to 2 ± 0.56 (Kirulapone) for indoors and from 0 (Imbulgoda, Keselwaththa and Thalwaththa) to 3.17 ± 1.6 (Dematagoda) for outdoors (Table 2). For *Ae. albopictus*, the mean larval count per ovitrap, in indoor and outdoor sites ranged from 0 (Kurana) to 1.56 ± 0.91 (Horana) and 0 (Grandpass) to 3.14 ± 0.51 (Horana), respectively (Table 2). Both species showed significant positive correlations between the mean larval count per ovitrap and the respective ovitrap index in both indoor and outdoor sites (r ranging from 0.62 to 0.99, $p < 0.05$) (Figure 1, a-d).

In outdoor ovitraps, a significant increase in the mean number of larvae was observed for *Ae. albopictus* in Imbulgoda, Horana, Kalamulla, Peradeniya and Edanduwawa while in Kurana the value was significantly higher for *Ae. aegypti* ($p < 0.05$). However, a significant rise in the mean number of *Ae. aegypti* larvae per ovitrap for indoor sites compared to outdoor sites was observed in Keselwaththa only ($p < 0.05$). *Ae. albopictus* showed an increased mean number of larvae per ovitrap in Kirulapone and Grandpass for indoors and in Kurana and Edanduwawa for outdoors ($p < 0.05$) (Figure 2).

Analyses of weather parameters on the mosquito prevalence showed that OI for the two species has not been significantly affected by the subtle changes in temperature of the study sites since they are all located within the wet zone ($p > 0.05$). However, significant negative correlations with the rainfall were observed for outdoor *Ae. aegypti* OI ($r = -0.579$, $p < 0.05$) and for indoor *Ae. albopictus* OI ($r = -0.541$, $p < 0.05$) (Figure 3, b & c).

Additionally, no significant difference ($p < 0.05$) was observed between the species with regard to the mean OI for each district a result which was consistent across all four districts. Nevertheless, the mean Ovitrap Index of *Ae. aegypti* and the number of dengue cases reported during the surveillance period in each district showed a significant strong correlation ($r = 0.962$, $p < 0.05$), while no such correlation was observed for *Ae. albopictus* ($r = -0.255$, $p > 0.05$) (Figure 4, a & b).

Figure 3: Relationship between rainfall and Ovitrap Index for the two species for indoor and outdoor locations. (a) *Ae. aegypti* at indoor sites (b) *Ae. aegypti* at outdoor sites (c) *Ae. albopictus* at indoor sites (d) *Ae. albopictus* at outdoor sites (r and p values are from regression).

Figure 4: Relationship between Ovitraps Index averaged over the study sites within each district and number of dengue cases reported during the surveillance period in the district. (a) *Ae. aegypti* (b) *Ae. albopictus* (r and p values are from regression).

4. Discussion

The present study has shown that the majority of positive ovitraps were infested with *Aedes* mosquitoes, while all other species occupied less than 3% reconfirming the effectiveness of the ovitraps surveillance method in estimating the prevalence of dengue vectors. Since ovitraps surveillance allows to identify the dengue vector species that is predominant in the given surveyed area, it might be possible to predict which species would contribute more to any prevailing dengue outbreaks providing an opportunity to customize the control methods to suit the vector species. In addition, ovitraps surveillance could be used as a tool for assessing the effectiveness of the present vector control strategies in terms of the vector population density. For example, the Colombo district where extensive mosquito control campaigns are being implemented throughout the year had the highest *Aedes* population density among our study sites and recorded the highest dengue incidence rate. While exhibiting the versatility of ovitraps surveillance, this situation urges the respective authorities to look for more effective vector control strategies to contain dengue transmission.

According to Tham (2000), an Ovitraps Index above 10% for *Aedes* species in an area may indicate a possible risk of dengue outbreak. In the current study, all the surveyed areas exhibited an OI greater than 10% for either or both *Aedes* species inside or/ and outside. Among them, areas within Colombo which showed the highest OIs for both species, experience dengue outbreaks every two to three years, with highest number of dengue incidence in the country (Sirisena & Noordeen, 2014). Our study revealed that sub urban and rural areas are also under a threat of dengue outbreaks, and when favorable conditions prevail, they might progress towards an epidemic. This suggests that the studied areas irrespective of the level of urbanization, would need immediate attention in combating the rising mosquito populations.

The Container Index or the percentage of ovitraps positive for either of the two *Aedes* species was higher for outdoors compared to indoors implying their preference. The ovitraps indices calculated for the two species separately, confirms this observation, where out of the total 14

areas studied. OI for outdoor ovitraps was higher in 12 areas compared to indoor ovitraps for *Ae. albopictus* and for nine areas for *Ae. aegypti* (*Ae. aegypti* was absent in one area). Similarly, when the mean larval counts are considered, the values obtained per outdoor ovitrap was higher than that of indoor ovitrap for both species, further corroborating the observation that a large number of *Ae. aegypti* and *Ae. albopictus* females are ovipositing outdoors compared to indoors. This finding agrees with a similar study conducted in Ragama, Gampaha District, Sri Lanka where the average number of mosquito eggs and immature stages of both *Aedes* species in ovitraps kept outdoors were relatively higher than those placed inside houses (Wiiegunawardana et al., 2019). Similarly, another study done in Nepal indicated significantly higher occurrence of *Ae. aegypti* and *Ae. albopictus* or both showing coexistence, in outdoor containers compared to those placed indoors (Dhimal et al., 2015). Further, a similar larval survey carried out in Thailand also demonstrated that *Ae. aegypti* and *Ae. albopictus* exist more abundantly in outdoors compared to indoors (Chareonvirivaphap et al., 2001). Another study conducted in Kenya covering three major cities, has also reported the same observation with respect to *Ae. aegypti* (Agha et al., 2017).

Our finding of having a relatively higher ovitrap index for *Ae. aegypti* at outdoor areas contradicts with the general belief of the species being more endophilic. However, on the other hand, this inclination towards outdoor breeding habitats might only reflect the presence of more suitable environment parameters in outdoors than indoors for oviposition. According to Wong et al. (2011) gravid *Ae. aegypti* females show preference for laying eggs in ovitraps located outside and exposed to sunlight ($\geq 20\%$ of the day). Further, the rate of development of *Ae. aegypti* larvae was shown to increase significantly with the increase in water

temperature of container from 15 to 35°C (Tun-Lin et al., 2000). In addition, *Ae. aegypti* breeding in outdoor containers on an enormous scale with heterogeneous immature populations as observed by Saifur et al. (2012) provide further evidence for the adaptation of *Ae. aegypti* towards outdoor breeding. It is also plausible to assume that this outdoor breeding habit has evolved independent of their endophilic nature. For instance, the outdoor areas that were focused on during the study were immediate neighborhoods of the houses/buildings, where the mosquitoes would still have adequate contacts with humans, i.e. Thumulla area where no *Ae. aegypti* was found in indoor ovitraps, is an urbanized university premises located in the center of Colombo where more than 3000 people move around all day. Thus, the findings of the present study emphasize the importance of concentrating on the removal of outdoor breeding habitats in dengue control, despite the well-known endophilic nature of the mosquito.

Considering study sites individually, the abundance of *Ae. aegypti* was significantly higher compared to *Ae. albopictus* in Kurana in both indoor and outdoor sampling sites. Kurana is a sub urban coastal area adjoining the lagoon where the fishery industry is predominant. The houses located along the border of the lagoon are built close to the lagoon and as a consequence, the lagoon is exploited by the inhabitants. For example, many houses dispose garbage directly to the lagoon and unused/broken fishing boats and other fishing gear are strewn around providing ample sites for mosquito breeding. Since *Ae. aegypti* is well known for its preference for breed in artificial breeding sites near human dwellings (Guillena et al., 2010; Higa, 2011), this very likely provides an explanation for the higher prevalence of *Ae. aegypti* in Kurana over *Ae. albopictus* which tends to breed more in natural containers. Further, Ramasamy et al. (2011) has showed that *Ae. aegypti* and *Ae. albopictus*

larvae eggs and their larvae survive to emerge as adults in brackish water with salinity levels of 2 to 15 ppt in coastal peri-urban environments in Jaffna, Sri Lanka, indicating that brackish water does not hinder oviposition and larval development of these mosquito species. This observation is also in accordance with a previous study that reported high numbers of *Ae. aegypti* larvae both indoors and outdoors in the coastal town of Malindi, Kenya (Midega et al. 2006).

Ae. albopictus is believed to be more common than *Ae. aegypti* in rural and sub urban areas where dense vegetation is present (Dame, 1994). In the current study, *Ae. albopictus* was found to be significantly abundant in terms of both the Ovitrap Index and the mean larval count per ovitrap than *Ae. aegypti* in two of four rural areas; Imbulgoda and Kalamulla in both indoor and outdoor premises and in two of five sub urban areas; Horana and Edanduwwa in outdoor habitats. Similarly, another study conducted in four sub urban areas in Kandy and Kurunegala in Sri Lanka indicated a high abundance of *Ae. albopictus* over *Ae. aegypti* (Weeraratne et al., 2013).

However, both species did not show a significant difference in abundance in terms of OI in most of the areas i.e. eight of a total of 14 areas, including all four urban areas: Kirulapone, Dematagoda, Grandpass and Thummulla; two rural areas: Hanthana Road and Nawalapitiya and two sub urban areas: Thalwaththa and Peradeniya, in either of indoor and outdoor surveillances. This indicates that both *Ae. aegypti* and *Ae. albopictus* have competitively infested most of the sites studied, specifically in all urban areas where the dengue prevalence is high. Further, this denotes that *Ae. albopictus* is present at comparable numbers to *Ae. aegypti* in the urban areas, despite its well-known nature to prefer more rural and sub urban areas. In the current study, *Ae. albopictus* also did not show a

significant difference in prevalence among the urban, sub urban or rural areas either in indoor or outdoor surveillances, indicating successful existence of *Ae. albopictus* irrespective of the level of urbanization. Similar observations were also reported from elsewhere i.e. a study conducted in China reported heightened densities of *Ae. albopictus* in urban cities than in sub urban and rural areas in Guangzhou, China (Li et al., 2014) while a study in Sarawak, Malaysia reported significantly higher Ovitrap Index for *Ae. albopictus* in urban residential areas compared to rural, sub urban and remote residential areas. Furthermore, in the current study, *Ae. albopictus* has shown to compete for the oviposition sites in urban areas with *Ae. aegypti* which is considered to be the dominant vector in urbanized areas. These observations may suggest an ecological and behavioral change in breeding patterns of *Ae. albopictus* with increasing invasion of urban areas with less vegetation.

Ae. aegypti and *Ae. albopictus* are related species that occupy similar ecological niches (Klowden, 1993). In the current study, mixed breeding of *Ae. aegypti* and *Ae. albopictus* was observed in both indoor and outdoor ovitraps regardless of the level of urbanization of the sampling site. However, generally higher tendency of shared ovitraps was detected for outdoor habitats compared to those indoors. Further, the high abundance of *Ae. aegypti* larvae over *Ae. albopictus* in the shared containers may indicate *Ae. aegypti* to be more competitive than *Ae. albopictus*. A similar finding was observed in a previous study where *Ae. aegypti* was found at a higher frequency than *Ae. albopictus* in mixed bred containers (Chen et al., 2006).

Usually the number of larvae increases after one or two weeks of rain with the egg hatching simulated during the rain and resulting in an increase in the adult mosquito population. However, in the

current study, *Ae. aegypti* populations captured in outdoor ovitraps and *Ae. albopictus* captured in indoor ovitraps, were found to be inversely correlated with the rainfall. This might probably be due to the less competition of gravid mosquitoes for ovitraps, specially in indoor ones, caused by the numerous breeding sites created in the environment during rainy season or it might also be that the rain flushed the eggs and larvae out of the ovitraps that were kept outdoors. Similar results have been reported in a previous study where a negative association was observed between the Ovitrap Index of the dengue vectors and rainfall (Weeraratne *et al.*, 2013).

Despite these observations, the present study suffers from the limitation of not having a synchronized sample collection throughout the study sites. This might have affected the reliability of some of the observations made during the study, as mosquito abundance tends to vary throughout the year with the climatic and environmental changes. Notwithstanding this limitation, the two dengue vectors were comparable in their abundance in the four districts studied here. However, only *Ae. aegypti* was found to be significantly correlated with the dengue incidence indicating its substantial contribution to dengue transmission. This observation supports the idea of *Ae. aegypti* acting as the primary vector in transmitting the dengue virus in Sri Lanka. In contrast, *Ae. albopictus*, despite its relatively equal abundance to *Ae. aegypti*, may be acting as a maintenance vector.

5. Conclusion

Our findings point at a possible behavioral plasticity of *Ae. albopictus* to successfully infest all urban, semi urban and rural areas. However, despite their increased abundance and distribution, our results suggest that *Ae. albopictus*, has

not significantly affected the dengue incidences in the studied areas. Along with these observations, the present study emphasizes the possibility of using ovitrap surveillances as a sensitive indicator for estimating the size of the dengue vector population which could aid in identifying potential dengue outbreaks in the country.

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