Original Research

Analysis of Aedes Mosquito Ovitrap Surveillance Data and Reported Dengue Cases in Selected High-Risk Localities in the Kegalle District, Sri Lanka

T. K. C. Wickramasinghe[1*], S. N. Weerakoon[2], T. Ranathunga[3], A. Perera[4], A. S. J. Bandara[1]

[1]*Regional Director of Health Services Office, Kegalle*

[2]*Regional Director of Health Services office, Badulla*

[3]*Department of Zoology, Faculty of Science, Eastern University of Sri Lanka*

[4]*Regional Director of Health Services office, Nuwara Eliya*

[*]*Correspondence: krishan.chathu@gmail.com*

https://doi.org/10.4038/sljhr.v4i1.73

https://orcid.org/0000-0002-4797-3972

Abstract

Background: The primary and secondary vectors for dengue, Aedes aegypti Linnaeus and Aedes albopictus Skuse, necessitate rigorous vector surveillance to understand real-time vector population dynamics. While larval surveys are commonly employed, they alone do not adequately predict the risk of dengue transmission. The ovitrap survey is a useful technique that has demonstrated effectiveness in dengue control programs as a surveillance component; however, it is underutilized in dengue risk determination. Hence, it is crucial to assess their use in monitoring disease morbidity and forecasting dengue risk to prevent epidemics in dengue risk localities.

General Objective: This study evaluated Aedes ovitap surveillance data with disease surveillance data to assess the dengue transmission risk in selected localities in the Kegalle district.

Methods: Ovitrap surveillance was conducted monthly, from January 2023 to December 2023, in three high-risk localities in selected high-risk Medical Officer of Health (MOH) areas, namely, Mawanella, Dehiovita, and Warakapola. In each locality, a total of 100 stranded ovitraps were placed, 50 indoors and 50 outdoors, collected after five days, and examined for emerging larvae. Confirmed dengue case data were obtained from the web-based disease surveillance system, DENSYS, of the Ministry of Health, Sri Lanka. The Mean Egg Cup Density (MECD), Egg Hatched Rate (EHR), and Larval Count (LC) of Ae. aegypti and Ae. albopictus indoors and outdoors was measured for each MOH area. Their combined effects were analyzed using General Linear Model (GLM) ANOVA. The correlations between dengue cases and MECD, EHR, and LC for both mosquito species were tested using Spearman's rank correlation.

Results: Significant differences in MECD were observed between months ($P=0.006$), MOH ($P<0.05$), and Trap Placement (TP) ($P=0.05$). For MECD, there were significant influences from the combined effects of month*MOH ($P<0.05$) and MOH *TP ($P=0.05$). LC was also significantly affected by MOH ($P<0.05$), mosquito species ($P<0.05$), and the interaction of TP with mosquito species ($P<0.05$ The mean number of dengue cases showed significant correlations with the MECD ($P=0.020$ and LC of Ae. albopictus ($P=0.011$), Indoor EHR ($P=0.013$), and Outdoor EHR ($P = 0.017$).

Conclusion: The positive relationship between the outcome of the ovitrap surveillance data and the dengue case data highlights the importance of integrating both datasets to monitor dengue morbidity and Aedes populations as well as to effectively forecast dengue epidemics in dengue high-risk localities,

Keywords: Aedes aegypti; Aedes albopictus; ovitrap surveillance; Dengue

Background

Aedes aegypti Linnaeus and Aedes albopictus Skuse are the primary and secondary vectors of dengue virus transmission. In addition, both mosquito species are responsible for the transmission of yellow fever and chikungunya viruses in tropical and subtropical regions

of the world [1]. With environmental and anthropogenic activities, the rapid population expansion of dengue vectors has been observed in recent years, causing severe damage to the public health system [2]. Both species have this ability to distribute, owing to their adaptability to a vast range of environmental factors and anthropogenic activities [3].

Vector surveillance of medically important arthropod species is mandatory to access the real-time vector population status [4]. With no approved vaccines against the dengue virus monitoring and controlling mosquito vectors are important in means of controlling dengue fever [5] [6]. Evidence-based decision making on vector control strategies with the objective of maintaining dengue vector populations below or close to the minimum epidemic threshold level will have a promising result in slowing dengue virus transmission [7].

Mosquito-based surveillance focuses mainly on the collection of specimens, including eggs, larvae, pupae, or adults [8]. With the traditional immature surveys, ovitrap survey is a simple and most widely used surveillance tool for dengue vector sampling [4]. Ovitrap is a small black plastic container that is partly filled with water, and the internal walls are lined with filter paper for females to lay eggs. The presence and abundance of mosquitoes are indirectly estimated by counting the eggs [3]. Aedes surveillance using ovitraps is one of the most cost-effective and important tools for dengue control [9]. Ovitraps are a simple and convenient tool for Aedes surveillance. Previous studies have indicated that ovitrap surveillance could be used to predict dengue outbreaks, especially in areas of low Aedes infestation, and has been recommended as a surveillance tool for dengue control [9].

Currently, larval surveys are the most widely used surveillance technique in dengue control programs in the country. However, larval indices alone are inadequate indicators for predicting dengue transmission risk due to the biological specificities of some Aedes species and the cryptic nature of their breeding sites of dengue vectors [10]. Therefore, a combination of methods/tools is currently practiced simultaneously to monitor Aedes population dynamics and forecast dengue epidemics in many countries [11], [12].

Objective

This study evaluated Aedes ovitap surveillance data with disease surveillance data to assess the dengue transmission risk in selected localities in the Kegalle district.

Methods

Ovitrap surveys were conducted from January 2023 to December 2023 in three high-risk MOH areas (Mawanella, Dehiovita, and Warakapola) declared by the National Dengue Control Unit Sri Lanka. In each MOH, a high-risk locality was selected as the sentinel site. Selected Grama Niladhari (GN) divisions to place ovitraps are Kiringadeniya in Mawanella MOH, Thalduwa in Dehiovita MOH and Ambepussa in Warakapola MOH areas (Figure 1).

Stranded ovitraps (black colored plastic cups/cup capacity 350 ml, diameter 10 cm, inner surface lined with filter paper) (n= 100) were placed both indoors and outdoors, following standard operational procedures [13]. Here, the filter paper-lined ovitraps were filled with de-chlorinated tap water up to 1/3 of the filter paper and placed in selected premises. Ovitraps were collected after 5 days and transferred to the regional entomology laboratory in the Kegalle Regional Director of the Health Services (RDHS) Office. The egg paddles were kept to emerge larvae and then identified using the Amarasinghe.,1995 mosquito identification key [14], [15].

Ovitrap surveys were conducted monthly at each sampling locality. Fifty cups were placed inside the 50 houses, while the other 50 were kept outside (gardens of the respective houses). Cups were inspected and collected after 4 days of placement. The number of eggs on the filter paper was counted using a hand lance and recorded. The cups were transported back to the entomology lab of the RDHS Office, Kegalle, filled with dechlorinated tap water, and allowed to hatch. After emerging, the number of larvae was counted and each larva was identified up to the species level, using a compound microscope (X40) using larval characters such as the morphology of comb scales of abdominal segment; Egg count, LC with respective to the species were recorded in ovitrap data recording format introduced by National Dengue Control Unit.

Dengue case data were collected from the web-based disease surveillance of the dengue sentinel site, DENSYS of the Epidemiology Unit of the Ministry of Health, Sri Lanka; only confirmed cases were considered for the analysis.

In the data analysis, a General Linear Model (GLM) ANOVA was used to compare the MECD and LC of Ae. aegypti and Ae. albopictus mosquitoes, considering the factors of month, MOH, TP (i.e. indoors/outdoors), along with the combined effects of these factors. Correlations were analyzed using the Spearman rank correlation test between the calculated variables (i.e., MECD, LC, EHR) and the reported dengue cases. The level of significance was set at $\alpha = 0.05$. Before the analysis, the data were transformed into LogX+1 after performing the Anderson-Darling normality test. All statistical analyses were performed using the MINITAB 21 statistical software package.

Results

The results of the GLM ANOVA analysis indicated that MECD showed statistically significant differences in relation to the factors studied, including the month of observation ($P=0.006$), MOH ($P<0.05$), and TP ($P<0.05$). The GLM ANOVA results further indicated a significant combined effect of month × MOH on MECD ($P<0.05$). Similarly, the combined effect of MOH*TP significantly influenced the MECD ($P<0.05$). This model also demonstrated a strong fit to the data, with an R2 value of 92.9%; a summary of the test results is shown in Table 1.

Comparatively, the lowest MECDs were recorded in the Dehiovita MOH. The month of May specifically witnessed the lowest MECD in Dehiovita MOH area, with a value of 42.0±16.5. Conversely, the maximum MECD was recorded in May in the Mawanella MOH region, reaching 562.0±121.0. Dehiovita and Warakapola MOH areas showed the highest MECD, with peaks in July (183.0±50.0) and April (521.0±129.0), respectively. In Mawanella MOH, the lowest MECD was recorded in August (59.0±22.0). Compared with the Dehiovita and Warakapola MOH areas, the total number of dengue cases was relatively higher in the Mawanella MOH area. Throughout the year, the highest number of dengue cases (n=99) was reported in January in the Mawanella MOH area. A summary of the MECDs and the number of dengue cases is shown in Table 2. In addition, it was observed that MECDs were predominantly greater in outdoor locations during the majority of months, with the exception of March. However, peak MECD levels indoors were recorded in April, whereas peak MECD levels outdoors were recorded in May (Figure 2a).

According to the GLM ANOVA for LC, the mean LC of ovitraps was significantly different in relation to both the MOH ($P<0.05$) and species ($P<0.05$). Additionally, the LC showed statistical significance with the combined effects of MOH*Species ($P<0.05$), as well as the combined effect of TP*Species ($P=0.010$). However, there were no significant effects associated with the month, TP, combined effect of MOH*Species, or combined effect of TP*species on the LC. The GLM-ANOVA model demonstrated the best fit for the test results, with an R2 value of 76.5%. A summary of the test results is provided in Table 3.

The EHR of Ae. aegypti larvae is lower when compared to Ae. albopictus. There were no Ae. aegypti larvae emerged from egg cups in the Warakapola MOH area. The highest EHR of Ae. aegypti in Dehiovita and Mawanella MOH areas were recorded in January and May, respectively. The lowest EHR of Ae. aegypti in both the Dehiovita and Mawanella MOH areas were recorded in December (Figure 2b).

According to the ovitraps results, the occurrence of Ae. albopictus was consistent throughout the year compared to Ae. aegypti. The peak number of Ae. albopictus hatched larvae in the Warakapola MOH area were recorded in May, followed by April, August, and November. Similarly, in Mawanella, higher numbers of Ae. albopictus hatched larvae were recorded in January, February, and May (Figure 2c).

According to the correlation analysis, there was a significant correlation between the MECD and the mean number of dengue cases ($P=0.020$). Additionally, the mean number of hatched Ae. albopictus larvae (outdoor) exhibited a significant correlation with the mean number of dengue cases ($P=0.011$). Furthermore, the mean number of dengue cases showed significant relationships with indoor EHR ($P=0.013$) and outdoor EHR ($P=0.017$) (Table 4).

The trends of MECD and dengue cases exhibited a parallel pattern, with peaks occurring in May, August,

and November (Figure 3a). Although the trend was not statistically significant (Table 4), the emergence of Indoor and Outdoor Ae. aegypti larvae displayed peaks in January, May, and November, with the same peak pattern as the mean dengue cases (Figure 3b). By contrast, the mean number of hatched Ae. albopictus larvae showed an interrelated trend with the mean number of dengue cases (Figure 3c). Both indoor and outdoor EHRs exhibited a relatively strong and conspicuous trend pattern with the mean dengue cases (Figure 3d).

Discussion

Sampling of immature mosquito stages is a widely used technique in dengue entomological surveillance programs [4], [8]. Here, the outcome of the ovitraps surveys was tested in dengue cases. The MECD were high in Mawanella and Warakapola areas where the urbanized localities compared to Dehiovita area.

In the Mawanella MOH area, the MECD was elevated during the initial months of the year (January–February), the middle months of the year (May–July), and the final months of the year (November–December). A similar trend was noted for the occurrence of dengue cases in the Mawanella MOH area. In the Warakapola MOH area, MECD was observed during the middle months of the year (April-June), whereas in Dehiovita, MECD was recorded in the months of July-September. Despite this, there are three distinct MECD in each MOH area at the beginning, middle, and end of the year. However, the peak at the beginning of the year may be a continuation of the peak at the end of the previous year, resulting in two actual high MECD within a single year. Comparable outbreaks of dengue fever are also evident in the MOH regions of Warakapola and Dehiovita. Moreover, Sri Lanka typically experiences two annual epidemiological waves that coincide with the monsoonal rain pattern [16], [17].

Based on the species composition observed in the study area, Ae. aegypti was lower than that of the Ae. albopictus. Consequently, the MECD was higher in the outdoor locations than in the indoor locations. Previous studies have indicated that the Ae. albopictus predominantly inhabits outdoor environments [3]. Additionally, these outdoor populations of dengue mosquitoes tended to lay a greater number of eggs in May, coinciding with the peak of reported dengue cases. Although the Ae. aegypti is considered an indoor breeding mosquito, hatched larvae of Ae. aegypti were observed in both the indoor and outdoor ovitraps. Interestingly, the number of hatched Ae. aegypti larvae was particularly high in May, contributing to the significant outbreak occurred in that year. Consequently, the combined presence of high Ae. aegypti and outdoor Ae. albopictus populations had a positive effect on the occurrence of dengue outbreaks in May and November. Similar observations of the association between ovitrap indices and dengue cases have also been reported in Indonesia [9].

Following the peak of dengue in May, there was a significant decrease in the EHR for Ae. aegypti larvae, both indoors and outdoors. Nevertheless, there was a slight increase in the hatched larval number of Ae. aegypti in November. In contrast to Ae. aegypti, Ae. albopictus exhibited high EHR, with more larvae found outdoors than indoors. The peak in the outdoor EHR of Ae. albopictus may significantly contribute to the spread of the disease during the peak period of dengue.

According to the World Health Organization, ovitraps can be used to collect vector information, especially related to spatial availability and temporal patterns. In addition, they can be used to monitor the impact of vector control interventions [18]. Similarly, this study provides evidence for the variation in the spatial and temporal distribution of dengue vectors. Moreover, this study highlights the significant influence of ovitrap hatching rate on the occurrence of dengue outbreaks. The highest EHR overlapped with the peak of dengue cases, indicating that these vector species have a natural tendency to contribute positively to population expansion during periods of dengue outbreaks.

Conclusion and Recommendation

Aedes ovitrap surveys play a crucial role in determining the spatial and temporal distribution of dengue vectors. Moreover, the findings of these surveys were significantly correlated with the distribution of dengue cases. Specifically, the distribution of dengue cases exhibited correlations with ovitrap survey data, including MECD and EHR. Hence, ovitrap surveys, in conjunction with other methods, provide valuable insights into the dynamics of Ae. aegypti and Ae. albopictus populations, aiding in the assessment of dengue transmission risk.

Table 1: Summary of GLM ANOVA of ovi trap per cup egg density analysis

Factor	df	F-value	P-value
Month	11	3.46	0.006**
MOH	2	22.49	0.000**
Indoor/Outdoor	1	51.41	0.000**
Month*MOH	22	5.19	0.000**
MOH*Indoor/Outdoor	2	12.94	0.000**

**Significantly different at p=0.05, df - degree of freedom

Table 2: Mean per cup egg densities (MECD) with SE mean and dengue cases in three MOH areas (DE – Dehiovita, MW – Mawanella, WP – Warakapola) in each month of study period

Month	DE		MW		WP	
	MECD±SE mean	Dengue cases	MECD±SE mean	Dengue cases	MECD±SE mean	Dengue cases
January	183.0±50.0	31	398.0±161	99	172.0±113	27
February	143.5±36.5	23	345.5±50.5	51	132.0±94.0	33
March	153.0±1.0	29	196.0±2.0	50	64.0±15.0	13
April	168.5±35.5	38	170.0±14.0	36	521.0±129.0	19
May	42.0±16.5	52	562.0±121.0	77	436.0±276.0	50
June	86.5±21.5	36	236.5±40.5	57	267.0±189.0	49
July	246.0±2.0	34	303.5±44.5	49	158.0±50.0	36
August	187.5±3.5	28	59.0±22.0	38	442.0±333.0	46
September	100.0±20.5	13	214.5±2.5	31	119.0±87.0	19
October	62.0±41.0	27	156.5±48.5	24	129.0±91.0	28
November	89.5±7.5	44	313.0±97.0	61	309.0±162.0	66
December	67.5±37.5	39	234.0±113	32	259.0±150.0	28
Total eggs	3059		6375		7365	
Total cases		394		605		414

Table 3: Summary of GLM ANOVA comparison of hatched larvae in ovitraps

Factor	df	F-value	P-value
Month	11	1.67	0.089
MOH	2	21.12	0.000**
Indoor/Outdoor	1	2.62	0.109
Species	1	247.65	0.000**
Month*Species	11	1.34	0.211
MOH*Species	2	19.02	0.000**
Indoor/Outdoor*Species	1	6.93	0.010**

**Significantly different at p=0.05, df – degree of freedom

Table 4: Summary of Spearman rank correlation and significant P values (p=0.05) of the tested parameters. MECD – Mean per cup egg density, A – Ae.aegypti, B – Ae.albopictus

Parameters	Spearman rank correlation	P value
MECD (indoor) vs mean dengue cases	0.524	0.080
MECD (outdoor) vs mean dengue cases	0.657	0.020*
Mean A larvae (indoor) vs mean dengue cases	0.308	0.331
Mean A larvae (outdoor) vs mean dengue cases	0.484	0.110
Mean B larvae (indoor) vs mean dengue cases	0.399	0.199
Mean B larvae (outdoor) vs mean dengue cases	0.699	0.011*
Egg hatch rate (indoor) vs mean dengue cases	0.692	0.013*
Egg hatch rate (outdoor) vs mean dengue cases	0.671	0.017*

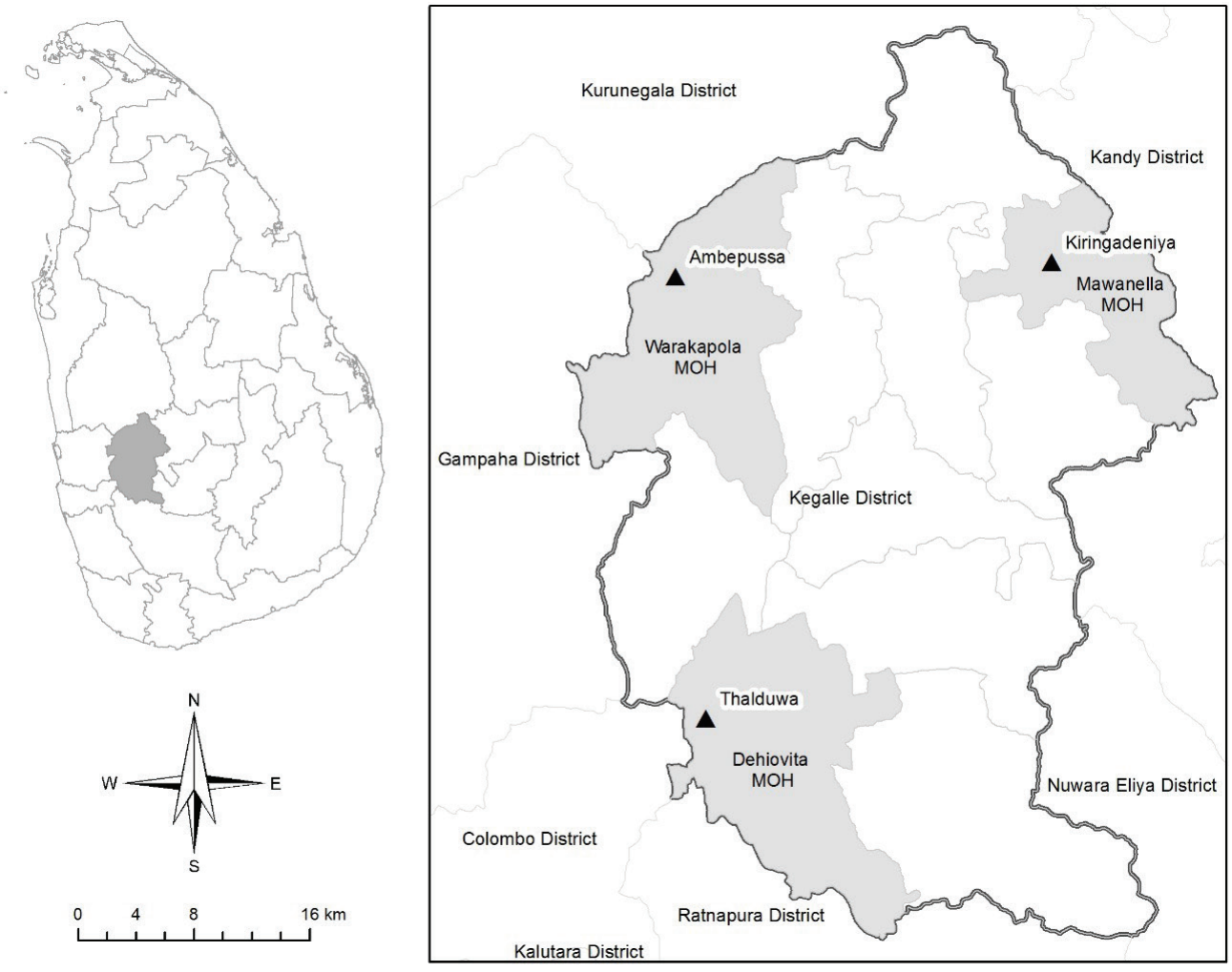

Figure 1: Map of the three study localities in Warakapola, Mawanella and Dehiovita MOH areas

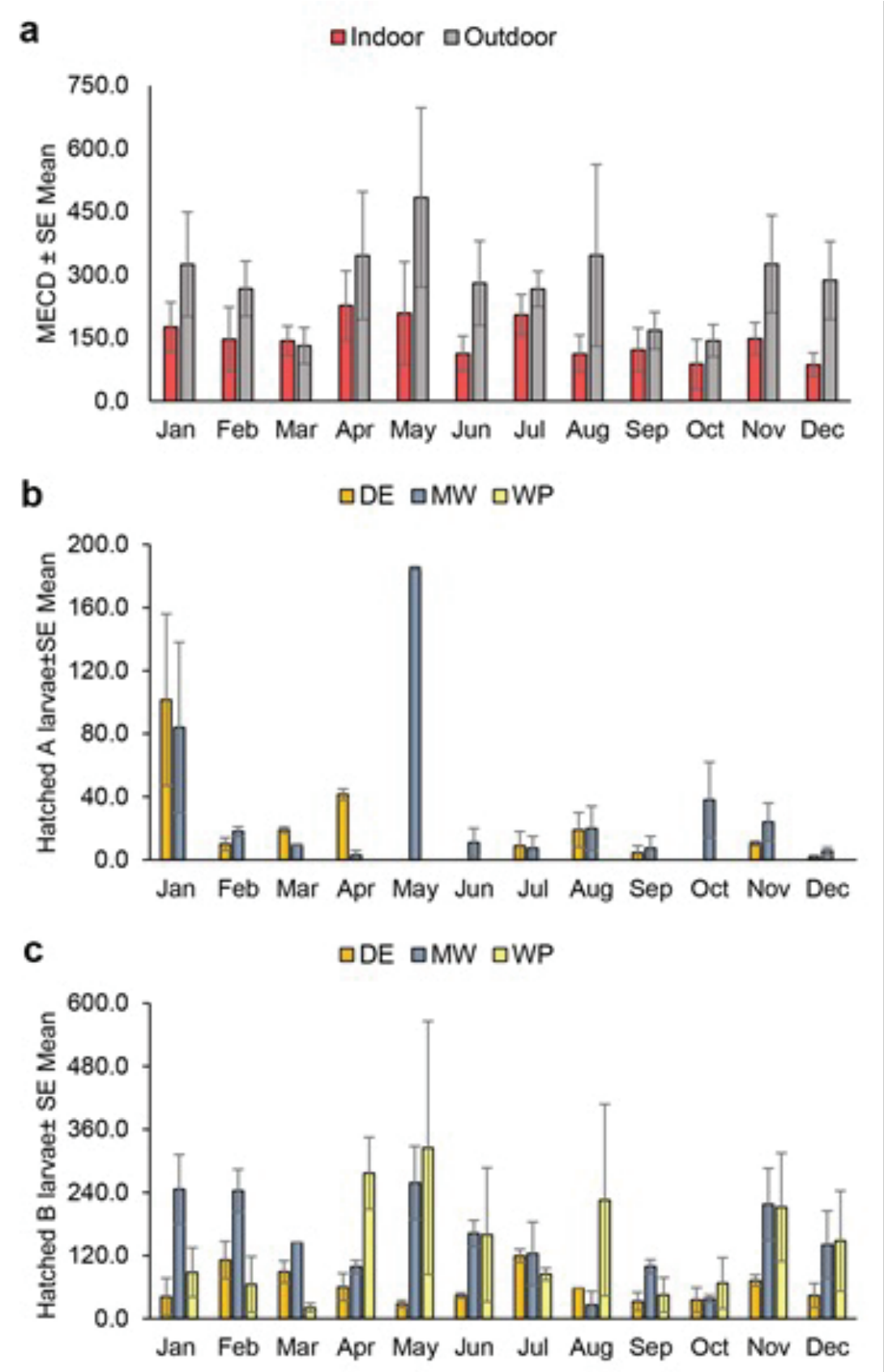

Figure 2: **Monthly variation of mean no of Mean per cup egg density (MECD) in indoors and outdoors (a), hatched A larvae (Ae.aegypti) of each MOH area (b) and hatched B larvae (Ae.albopictus) of each MOH (c). The short codes represent the MOH area, including DE – Dehiovita, MW – Mawanella, and WP – Warakapola.**

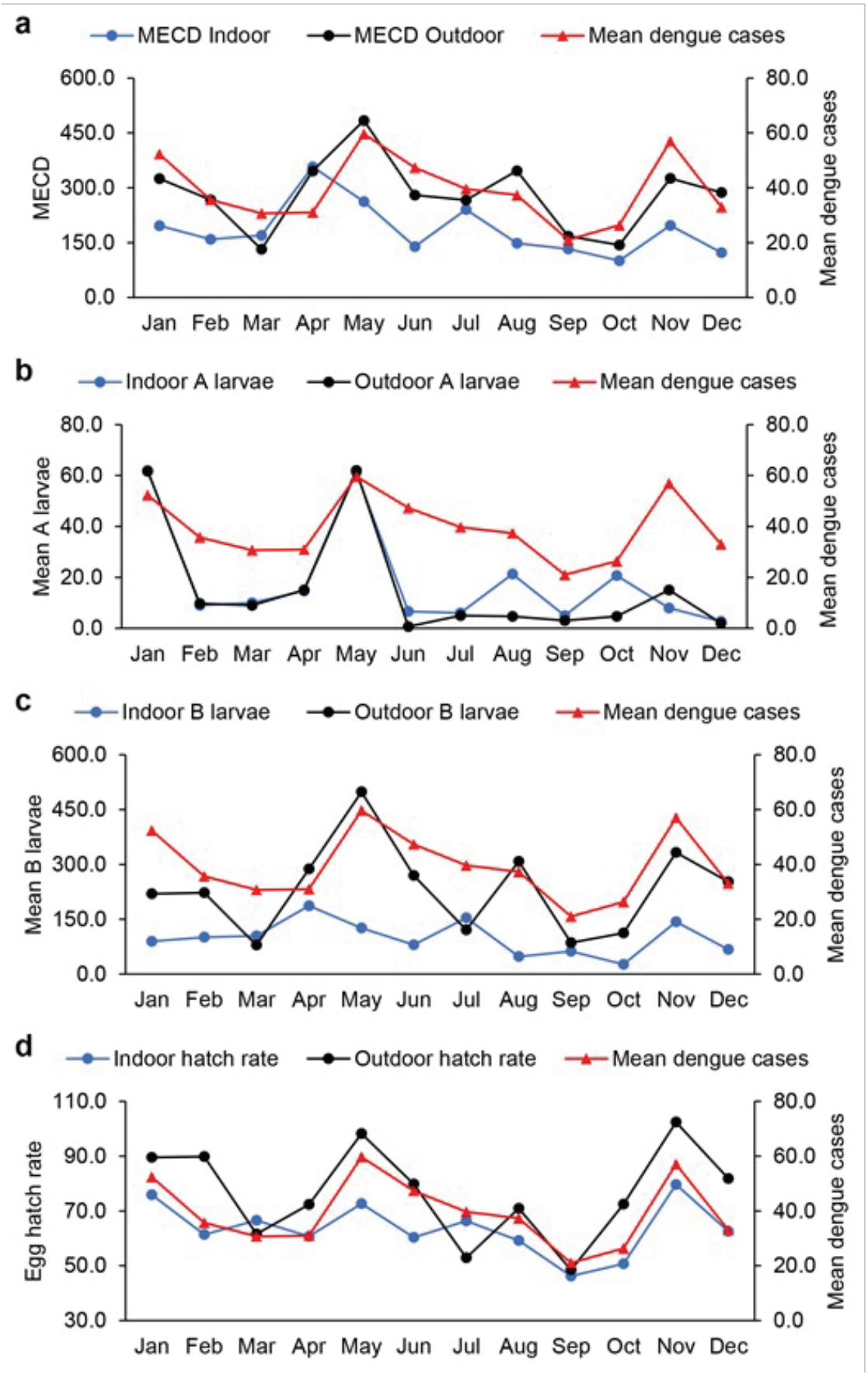

Figure 3: **Mean dengue cases trends with Mean per cup egg density (MECD) (a), Mean A larvae (b), Mean B larvae (c) and egg hatch rate (d). A – Ae.aegypti, B – Ae.albopictus**

Author declaration

Author contributions: All authors contributed to the conception or design of the work, data acquisition, analysis, or interpretation of data for the final manuscript drafting

Conflicts of interest: The authors declare no conflicts of interest concerning the research, authorship, and/or publication of this article

Ethics approval and consent to participate: The study was exempted from the review process by the Ethics Review Committee, National Institute of Health Sciences, Sri Lanka

Funding: This study did not receive grants from any funding agency in the public, commercial, or not-for-profit sectors.

References

[1]. Wu HH, Wang CY, Teng HJ, Lin C, Lu LC, Jian SW, et al. A dengue vector surveillance by human population-stratified ovitrap survey for adult Aedes (Diptera: Culicidae) and egg collections in high dengue-risk areas of Taiwan. J Med Entomol. 2013 Mar;50(2):261-9. doi: 10.1603/me11263. PMID: 23540112.

[2]. Gato R, Menéndez Z, Prieto E, Argilés R, Rodríguez M, Baldoquín W, et al. Sterile insect technique: Successful suppression of an Aedes aegypti field population in Cuba. Insects. 2021;12:469. doi: 10.3390/insects12050469.

[3]. Velo E, Kadriaj P, Mersini K, Shukullari A, Manxhari B, Simaku A, et al. Enhancement of Aedes albopictus collections by ovitrap and sticky adult trap. Parasit Vectors. 2016 Apr 21;9:223. doi: 10.1186/s13071-016-1501-x. PMID: 27102015; PMCID: PMC4839143.

[4]. Schaffner F, Bellini R, Petrić D, Scholte EJ, Zeller H, Marrama RL. Development of guidelines for the surveillance of invasive mosquitoes in Europe. Parasit Vectors. 2013;6:209.

[5]. Lambrechts L, Scott TW, Gubler DJ. Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. PLOS Negl Trop Dis. 2010;4(5):e646.

[6]. Wu T, Wu Z, Li YP. Dengue fever and dengue virus in the People's Republic of China. Rev Med Virol. 2022;32(1):e2245. doi: 10.1002/rmv.2245.

[7]. Wijegunawardana NDAD, Gunawardene YINS, Chandrasena TGAN, Dassanayake RS, Udayanga NWBAL, Abeyewickreme W. Evaluation of the effects of Aedes vector indices and climatic factors on dengue incidence in Gampaha District, Sri Lanka. Biomed Res Int. 2019 Jan 31;2019:2950216. doi: 10.1155/2019/2950216.

[8]. Djiappi-Tchamen B, Nana-Ndjangwo MS, Nchoutpouen E, Makoudjou I, Ngangue-Siewe IN, Talipouo A, et al. Aedes mosquito surveillance using ovitraps, sweep nets, and Biogent traps in the city of Yaoundé, Cameroon. Insects. 2022;13:793. doi: 10.3390/insects13090793.

[9]. Norzahira R, Hidayatulfathi O, Wong HM, Cheryl A, Firdaus R, Chew HS, et al. Ovitrap surveillance of the dengue vectors, Aedes aegypti and Aedes albopictus in selected areas in Bentong, Pahang, Malaysia. Trop Biomed. 2011 Apr;28(1):48-54. PMID: 21602768.

[10]. Sasmita HI, Neoh KB, Yusmalinar S, Anggraeni T, Chang NT, Putra RE, et al. Ovitrap surveillance of dengue vector mosquitoes in Bandung City, West Java Province, Indonesia. PLoS Negl Trop Dis. 2021;15(10):e0009896. doi: 10.1371/journal.pntd.0009896.

[11]. Hemme RR, Smith EA, Felix G, White BJ, Diaz-Garcia MI, Rodriguez D, et al. Multi-year mass-trapping with autocidal gravid ovitraps has limited influence on insecticide susceptibility in Aedes aegypti from Puerto Rico. J Med Entomol. 2022;59:314–9.

[12]. Zhou Y, Liu H, Leng P, Zhu J, Yao S, Zhu Y, et al. Analysis of the spatial distribution of Aedes albopictus in an urban area of Shanghai, China. Parasit Vectors. 2021;14:501.

[13]. National Dengue Control Unit, Ministry of Health Sri Lanka. Standard Operating Procedures for Aedes vector surveillance in Sri Lanka. 2019. ISBN: 978-955-3666-50-5.

[14]. Amarasinghe FP. Illustrated keys to the genera of mosquitoes (Diptera: Culicidae) in Sri Lanka. J Natn Sci Coun Sri Lanka. 1995;23(4):183-211.

[15]. National Dengue Control Unit, Ministry of Health Sri Lanka. Guidelines for Aedes vector surveillance and control in Sri Lanka. 2016. ISBN: 978-955-0505-85-2.

[16]. Prabodanie RAR, Stone L, Schreider S. Spatiotemporal patterns of dengue outbreaks in Sri Lanka. Infect Dis. 2020;52(5):350-60. doi: 10.1080/23744235.2020.1725108.

[17]. Sirisena PDNN, Noordeen F. Evolution of dengue in Sri Lanka—changes in the virus, vector, and climate. Int J Infect Dis. 2014;19:6-12.

[18]. Focks DA. A review of entomological sampling methods and indicators for dengue vectors. Dengue Bull. WHO Special Programme for Research and Training in Tropical Diseases; 2003.