PRIMARY MALARIA VECTOR *Anopheles culicifacies* (GILES): ABUNDANCE AND CLIMATIC CORRELATIONS IN A POST-MALARIA ELIMINATION SETTING

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INTRODUCTION

Malaria, a vector-borne disease, has been eliminated in Sri Lanka since 2012. However, there is a high risk of the re-establishment of malaria in the country due to increased receptivity and the importation risk. Receptivity for malaria is mainly due to the abundant presence of *Anopheles* vectors, and ecological and climatic factors favourable for malaria transmission. Since the 1920s, *Anopheles culicifacies* (Giles) is considered to be the primary malaria vector in Sri Lanka (Amerasingha *et al*., 1999).

In the past, malaria was endemic in the dry and intermediate zones, and an epidemic in the wet zone. The major factor that determined the distribution of malaria in Sri Lanka has been the presence of favourable climatic conditions for *An. culicifacies* breeding, Studies have revealed the presence of *An. culicifacies* in the dry and intermediate zones (Ramasamy *et al.*, 1994; Amerasinghe *et al.*, 1999; Gunathilaka *et al*., 2013; and Ranatunga *et al.*, 2020). However, evidence for the presence of *An. culicifacies* is limited in the wet zone where there is a higher importation risk for malaria. Therefore, this study was designed to assess the distribution and abundance of *An. culicifacies* across all climatic zones of Sri Lanka and to correlate climatic factors, rainfall, temperature, and relative humidity with densities of *An. culicifacies.* It was expected that the findings will be useful in determining receptivity in the efforts of sustaining the malaria eliminated status in the country.

METHODOLOGY

Study sites

The study was carried out in five sites representing the three climatic zones of Sri Lanka. Three sites were in the dry zone, which were in theHorowpothana MOH of the Anuradhapura District, Poonakary MOH area of the Kilinochchi District, and Buttala MOH area of the Moneragala District. The other two sites were the Nikaweratiya MOH of the Kurunegala District in the intermediate zone and the Meerigama MOH of the Gampaha District in the Wet zone. In each of the above MOH areas, two GN divisions were selected for the study considering historical evidence for malaria transmission. Climatic data was collected from the Department of Meteorology, Colombo on a monthly basis for the five study sites.

Sample collection methods

An. culicifacies mosquitoes were collected monthly, from April 2015 to March 2017. Adult females were sampled using Cattle-Baited Trap Collections (CBTC), Human Landing Catches (HLC), and indoor Hand Collections (IHC). Larval Surveys (LS) were carried out using the dipping technique to collect the immature stages of *An. culicifacies.* They were carried out following the Standard Operating Procedures (SOP) of the Anti-Malaria Campaign, Sri Lanka, and adult and larval specimens were identified for the species on external morphology using standard identification keys (Amerasinghe, 1990).

Data analysis

The mean densities for each adult trapping method was calculated by dividing the number of *An. culicifacies* from the total number of sampling efforts per month. The mean monthly density of larvae was determined by dividing the total number of larvae collected from the total number of larval dips and multiplying by 100. Adult and larval densities of *An. culicifacies* species derived from CBTC, HLC, HC, and LS from different study sites were compared using the Kruskal-Wallis H test with Dunn's post hoc test for multiple comparisons using SPSS 21. For each study site, the mean monthly rainfall, maximum temperature, minimum temperature, average temperature, and relative humidity data were correlated with monthly mean densities of *An. culicifacies* from each technique. Spearman's rank order correlation matrices were analyzed using the Past v 2.33 to investigate the correlation between monthly climatic factors and the monthly mean adult and larval densities of *An. culicifacies.*

RESULTS AND DISCUSSION

Abundance of *Anopheles culicifacies* in study sites

A total of 10,381 of *An. culicifacies* adults and larvae were collected by the four collection methods during 24 surveys. Table 1 shows the number of *An. culicifacies* collected from each collection method in each study site and the percentage abundance from the total collection.

Table 1. Total numbers (Percentage) of *An. culicifacies* collected from the study sites

Collection method	Total number of mosquitoes collected (Percentage)					Total
	Horowpothana	Poonakary	Buttala	Nikaweratiya	Meerigama	
CBTC (240 traps)	9 (2.15)	2 (0.48)	95 (22.73)	310 (74.16)	2 (0.48)	418
HLC (4495 man hours)	47 (3.21)	0	372 (25.38)	773 (52.73)	274 (18.69)	1466
HC (2425.25 man hours)	2 (1.60)	0	6 (4.80)	110 (88.00)	7 (5.60)	125
LS (131022 dips)	663 (7.92)	340 (4.06)	1315 (15.71)	5280 (63.07)	774 (9.25)	8372

The highest numbers of *An. culicifacies* specimens were collected from the intermediate zone of the Nikaweratiya site. In the Nikaweratiya site, the abundance percentage for CBTC was 74.16%. It was 52.73%, 88.00%, and 63.07% from HLC, HC, and LS, respectively. The second-highest abundance was from the Buttala site in the dry zone for CBTC (22.73%), HLC (25.38%), and LS (15.71%). However, the second-most abundance for HC was from the Meerigama site (5.60%) in the wet zone. The Poonakary site in the dry zone recorded the lowest abundance of *An. culicifacies* from CBTC and LS.

Further, densities of *An. culicifacies* were not similar between the five study sites from all collection methods from the Kruskal-Wallis H (df 4) statistic (CBTC: 43.2, $p<0.001$, HLC: 45.2, $p<0.001$, HC: 24.4, $p<0.001$, LS:15.4, $p<0.01$). The highest density of *An. culicifacies* in CBTC was found from the Nikaweratiya study site (6.46 adult females per trap) and it was significantly different from the Horowpothana, Poonakary, and Meerigama sites ($p<0.05$). In LS, highest density was in the Nikaweratiya site (29.54 larvae per 100 dips), which was significantly different from the Poonakary and Meerigama sites ($p<0.05$) (Figure 1). In HC, Nikaweratiya had the highest density of 0.21 adult females per man hour and it was significantly different from the other four sites ($p<0.05$). In HLC, the highest mean density of 0.63 adult females per man hour reported from Nikaweratiya was significantly different from that of the Horowpothana, Poonakary, and Meerigama sites ($p<0.05$). Of the other sites, Poonakary had the lowest densities and there were no adult females found in HLC and HC. Horowpothana recorded low levels of *An. culicifacies* densities in HLC (0.07 per man hour) and IHC (0.003 per

man hour) while the Buttala site recorded considerable levels of *An. culicifacies* densities in HLC (0.31 per man hour) and IHC (0.1 per man hour). Considerable densities of *An. culicifacies* in HLC (0.24 per man hour) and IHC (0.1 per man hour) in the Meerigama site suggest that even in the wet zone, there is a high risk of malaria transmission potential (Figure 2). The percentage abundance and mean density comparisons in this study suggest that *An. culicifacies* was found in all areas with more prominence in the intermediate zone for breeding as well as human biting and indoor resting populations. Moreover, it was evident that even within the dry zone, there are differences in distribution and abundance.

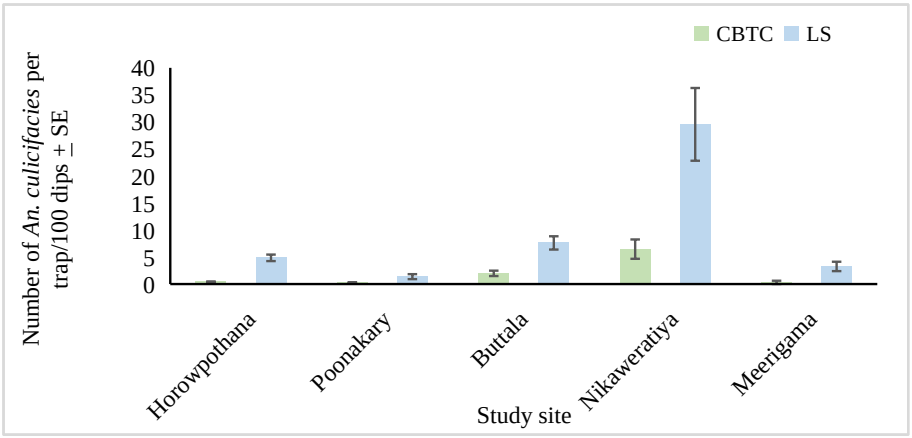

Figure 1. Mean densities of *An. culicifacies* in CBTC and LS in different study sites

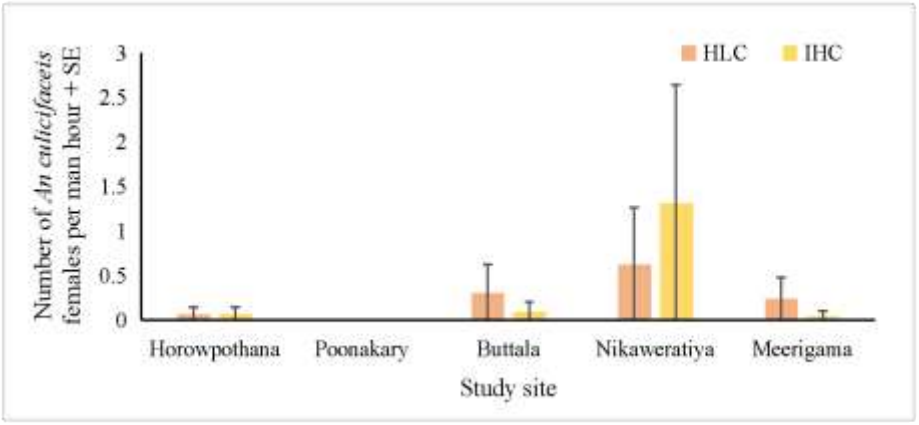

Figure 2. Mean densities of *An. culicifacies* in HLC and HC in different study sites

Correlations of *An. culicifacies* densities with climatic factors

It is understood that mosquito abundance and the distribution of mosquitoes depends on the role of the local climate, especially in areas like highlands and desserts. Therefore, a Spearmans' rank order correlation analysis was carried out to find the correlations of mean monthly densities of *An. culicifacies* with climatic parameters, and only the significant correlations at $p<0.05$ and $p<0.01$ are given in Table 2.

An. culicifacies densities were negatively correlated with rainfall. Adult densities from HC and HLC in Meerigama had a negative correlation with the current month's rainfall ($p<0.05$). *An. culicifacies* larval densities of Meerigama and thecurrent month rainfall also had a strong negative correlation ($p<0.01$). Larval densities of Nikaweratiya too had a negative correlation with 2 months' lag rainfall ($p<0.05$). *An. culicifacies* is prone to breed mainly in drying-up rivers, which depends on the pattern of rainfall; these findings support the evidence for high abundance of *An. culicifacies* in dry periods, especially in the wet and intermediate zones.

Temperature and *An. culicifacies* densities showed a significant negative correlation in Horowpothana (CBTC: Tavg, $p<0.05$), Buttala (HLC Tmax, $p<0.05$ & HC: Tmax, $p<0.05$), Nikaweratiya (HLC Tavg, $p<0.05$ & LS: Tavg, $p<0.05$), and Meerigama (CBTC: Tmin, $p<0.05$, Tavg, $p<0.05$, HLC: Tmin, $p<0.05$, IHC: Tmin, $p<0.01$, LS: Tmin, $p<0.01$). This observation suggests that high temperatures had reduced the abundance, especially in wet zone and in the two dry zone sites**.** Relative humidity had a significant negative correlation in the Meerigama site with indoor resting densities and larval densities. This suggests that the requirement of high humidity for indoor resting might have been met outdoors than indoors in the wet zone site (Table 2).

Table 2. Correlation between *An. culicifacie*s densities and climatic variables

Collection technique	**RF lag 0**	**RF lag 1**	**RF lag 2**	**Tmax**	**Tmin**	**Tavg**	**RH**
CBTC	NS	NS	NS	NS	-0.423*^{M}	-0.444*^{H} -0.423*^{M}	NS
HLC	-0.493*^{M}	NS	NS	-0.416*^{B}	-0.435*^{M}	-0.477*^{N}	NS
HC	-0.502*^{M}	NS	NS	-0.515*^{B}	-0.552**^{M}	NS	-0.554**^{M}
LS	-0.655**^{M}	NS	-0.403*^{N}	NS	-0.411*^{H} -0.623**^{M}	-0.443*^{N}	-0.539**^{M}

*Significant at p< 0.05, **Significant at p<0.01, NS - Not Significant
RF-Rain fall, Tmax- Maximum Temperature, Tmin- Minimum Temperature, Tavg-Average Temperature, RH-Relative Humidity, H-Horowpothana, P-Poonakary, B-Buttala, N-Nikaweratiya, M-Meerigama

CONCLUSIONS

Sri Lanka is experiencing 'anophelism without malaria' after malaria elimination from the country in 2012. It was observed that *An. culicifacies* is distributed in all climatic zones with a spatial variability of its abundance. Moreover, it was evident that three key climatic factors, rainfall, temperature, and relative humidity play a role in the distribution and abundance of *An. culicifacies* within the climatic zones. In future efforts for determining malaria receptivity, it is suggested to elicit the importance of climatic correlations with vector abundance.

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