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ANOPHELINE MOSQUITO FAUNA OF SRI LANKA: THEIR ABUNDANCE, ENVIRONMENTAL CORRELATES AND BIONOMICS IN MALARIA POST-ELIMINATION STAGE

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ABSTRACT

Although malaria was eliminated from Sri Lanka in 2012, the risk of re-establishment of the disease in the country is observed to be high due to the constant influx of imported malaria cases, presence of the species of malaria vector *Anopheles* and the favourable environmental conditions. This study was designed to understand the risks posed by *Anopheles* species to malaria re-establishment in the country. The main aim was to determine the occurrence, distribution, abundance, seasonality and bionomics of anopheline fauna, and their climatic correlates across a wide geographical range of the island. The study sites represented all three climatic zones: Horowpothana, Poonakary and Buttala in the dry zone, Nikaweratiya in the intermediate zone, highly endemic areas of the disease before elimination of malaria and Meerigama in wet zone, which is considered as non-endemic. World Health Organization recommended standard survey methods, namely Cattle Baited Trap Collections (CBTC), Larval Survey (LS), Human Landing Catch (HLC) & Indoor Hand Collection (IHC), were used in monthly sampling of larvae and adults of vector mosquitoes from April 2015 to March 2017. Data were analyzed using Kruskal Wallis test, Spearman's and Pearson's correlations and Canonical Correspondence Analysis (CCA) using PAST software to determine the distribution, abundance, environmental correlates, and their significance, and to understand the bionomics of anopheline mosquito species.

A total of 17 *Anopheles* species were recorded including the primary malaria vector, *Anopheles culicifacies* and ten secondary and potential malaria vectors mostly with a constant distribution and six non-malaria vectors mostly with infrequent or sporadic distribution. Nikaweratiya recorded significantly higher

($p<0.05$) mean densities of *An. culicifacies* adults and larvae from all methods ($p<0.05$). Densities of secondary and potential vectors significantly ($p<0.05$) varied among study sites. Increase of larval and adult densities of different species varied with monsoon rainfall pattern were evident for *An. culicifacies* in the absence of monsoon rains and for *An. subpictus* and *An. tessellatus* after rains, whereas for *An. vagus* during or soon after the rains as sudden spikes. *An. varuna* human biting and indoor resting densities showed a seasonality in dry zone site. Rainfall of current month (RFlag0) and previous two months (RFlag1 & RFlag2), average temperature (Tavg) and relative humidity (RH) were found to be the important climatic correlates that influence the densities of *Anopheles* species within study sites. Most secondary vectors had significant positive correlations ($p<0.05$) between larval and/or adult densities and rainfall. In contrast, *An. culicifacies* larvae in Nikaweratiya and Meerigama showed a negative significant correlation, which was confirmed by multivariate CCA plots. Thus, *An. culicifacies* breeding was not found to be dependent on rainfall unlike most secondary vectors.

Anopheles species were found breeding in a wide range of aquatic habitats, varying from very small water collections to larger water bodies and even water storage sites created by human activities. The most preferred larval habitat of *An. culicifacies* was river bed sand pools, which were present in four study sites, except Poonakary. In Poonakary, *An. culicifacies* had shifted its breeding to wells. Bi-variate correlations and multivariate CCA plots confirmed a positive association between *An. culicifacies* and *An. varuna* with the temperature of riverbed sand/rock pools and wells respectively. There was a positive correlation between *An. subpictus* and Total Suspended Solids (TSS)

in bi-variate analysis and CCA analysis showed a positive association with conductivity and Total Dissolved Solids (TDS) in lagoon margins, tank bed pools and marshy lands.

Anthropophagic behaviour was observed in 13 species of *Anopheles*, including two non-vector species. The highest species richness in terms of anthropophagy was restricted to the dry zone Buttala site. *An. culicifacies* was still the most human biting species with highest human biting rates (HBRs), both indoors and outdoors, as observed in Nikaweratiya. There were 10 (in door) resting *Anopheles* species, where *An. subpictus* was the most abundant species (0.95 per premise) followed by *An. culicifacies* (0.034 per premise). Hanging clothes were the preferred indoor resting surface of *An. culicifacies* (40%) and *An. subpictus* (76%).

The constant presence of primary, secondary and potential vectors in all study sites indicates that all selected study areas have a high receptivity to malaria transmission in the post-elimination period in Sri Lanka. Nikaweratiya stood out as an area highly conducive for malaria transmission; its perennial river system may have supported breeding and bionomics of the primary vector leading to the observed high densities of larvae and adults, high human biting and indoor resting rates. Significant new findings were on exophagic human biting behavior and alteration of indoor resting surfaces of malaria vectors, including of *An. culicifacies*.

Moreover, the study highlights the need of molecular techniques for accurate identification of species in systematic vector monitoring, since a morphologically identified *An. nigerrimus* belonging to Hyrcanus series could not be confirmed by molecular studies. Overall, this study describes how

abundance of vectors, availability of larval habitat types, prevailing climate and the environment interact in a given area, which could be used in future revisions of receptivity quantification efforts of the Ministry of Health, guiding the entomological surveillance and vector control for prevention of re-establishment (PoR) of malaria in Sri Lanka.