PREVALENCE AND BIONOMICS OF THE *Anopheles* MOSQUITOES IN SELECTED SITES IN THE HAMBANTOTA DISTRICT

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INTRODUCTION

Malaria, caused by parasites of the genus *Plasmodium* and transmitted by the female *Anopheles* mosquitoes, is a serious public health concern in many tropical and sub-tropical countries of the world (WHO, 2021). In Sri Lanka, malaria was endemic in 22 districts in the intermediate and dry zones of the country. The most devastating malaria outbreak recorded in Sri Lanka was during the 1934–35 period when close to 80,000 human deaths were reported. In September 2016, Sri Lanka received WHO certification for the elimination of malaria from the island, with the last indigenous case being reported in October 2012. Currently, the island is in the phase of the Prevention of Re-establishment (PoR) of malaria. However, Sri Lanka is vulnerable to reintroduction and transmission of malaria due to the continuous influx of imported malaria cases through returning residents, visitors, and the high abundance of malaria vectors in most parts of the country (Shretta *et al.*, 2017).

In Sri Lanka, the primary vector of malaria is reported to be the *An. culicifacies.* The *An. subpictus, An. annularis, An. varuna, An. vagus*, and *An. tessellatus* are considered as secondary vectors of malaria (AMC, 2020). The *An. stephensi* is considered an invasive malaria mosquito species (Darmasiri *et al.,* 2017). Up to now, 24 anopheline species have been reported from Sri Lanka (Gunathilaka *et al*., 2015).

Hambantota is one of the most vulnerable districts in the country for malaria with the presence of a harbour, airport, and many other tourist attractions. There may also be illegal entry points in the district as it is located along the coast. Hence, there is a risk of the introduction of malaria parasites via these entry points to Sri Lanka. Furthermore, a better understanding of the current status on *Anopheles* prevalence, seasonal changes, bionomics, insecticide resistance status, and resistance mechanisms is required to initiate a successful vector control management programme in Hambantota. Despite the risk of the introduction of malaria and the importance of having a good understanding of vectors that could transmit malaria, there are no published reports on the *Anopheles* mosquitoes in Hambantota. Thus , the aim of this study was to examine the prevalence and bionomics of *Anopheles* in Hambantota as this information is crucial when taking malaria vector control decisions. Specifically, it examined the species richness, relative abundance, species diversity, breeding habitats, and biting behaviour of *Anopheles* in the selected sites in the District of Hambantota over a period of six months.

METHODOLOGY

Arabokka, Mirijjawila, and the Harbour localities in the Hambantota Medical Officer of Health (MOH) area, and the Mattala locality in the Lunugamvehera MOH area were selected as the study sites. Entomological surveys comprising Larval Surveys (LS), Cattle-Baited Trap Collections (CBTC), and Cattle-Baited Hut Collections (CBHC) were carried out monthly in selected sites from October 2021 to March 2022, as per the standard operational procedures of the Anti-Malaria Campaign (AMC, 2016). The secondary data of the Human Landing Night Collections (HLNC) in the same localities were obtained from the AMC for the study period. The mosquito samples were transported to the laboratory and were identified morphologically using published keys (Amarasinghe, 1990; Amarasinghe, 1992).

The relative abundance of the species was expressed as the percentage of the total number of *Anopheles* collected. Species abundance in the selected localities were compared in the study sites with Oneway ANOVA using SPSS 28.0.1 Version. Species richness and diversity in the different localities were analysed using the Shannon Weiner Diversity index.

RESULTS AND DISCUSSION

Prevalence of *Anopheles*

A total of 7072 mosquitoes (larvae 829, adults 6243) belonging to 11 *Anopheles* species were recorded from the four localities during the study period. Shannon Wiener species diversity indices based on CBTC collections varied among the localities: Mattala 1.86, Mirijjawila 1.46, and Arabokka 1.3. A total of 829 *Anopheles* larvae belonging to 10 species were collected from the LS carried out in Arabokka, Mirijjawila, the Harbour and Mattala localities. This total comprised 255 (30.76%) *An. subpictus*, 169 (20.39%) *An. nigerrimus*, 103 (12.42%) *An. varuna*, 94 (11.34%) *An. culicifacies*, 72 (8.69%) *An. peditaneatus*, 63 (7.6%) *An. barbirostris*, 39 (4.7%) *An. vagus*, 29 (3.5%) *An. pallidus*, 3 (0.36%) *An. jamesii*, and 2 (0.24%) *An. tessellatus* mosquito larvae. *Anopheles* larvae were more abundant in the Mattala study site (333, 40.2%), followed by the Harbour locality (257, 31%), Mirijjawila (155, 18.7%), and Arabokka (84, 10.1%), respectively. The highest and lowest species abundance of the *An. subpictus* larvae were reported in Mirijjawila (107 (69%)) and Mattala (34 (10.2%)), respectively. The highest and lowest species abundance of *An. culicifacies* larvae were reported in Mattala (58 (17.4%)) and Arabokka (0%), respectively.

The *An. subpictus* (identified as B complex based on Suguna *et al*., 1994) was the most predominant larval stage in Arabokka, Mirijjawila, and the Harbour sites. The Species B complex, known to be the predominate sibling species in coastal areas (Jude *et al.*, 2013), is an efficient vector of malaria in Sri Lanka (Abhayawardana *et al.*, 1996). With high abundance of the secondary vector, the *An. subpictus,* and the presence of the primary vector, *An. culicifacies*, the Hambantota and Lunugamvehera MOH areas can be considered as being highly receptive for malaria.

The most predominate *Anopheles* larval species found in the Mattala site was the *An. varuna*. Mattala, located towards the inland of the District of Hambantota, showed the highest larval species richness and diversity compared with other coastal sites. The availability of the high number of permanent breeding habitats such as canals, streams, tanks wells, and other man-made mosquito breeding habitats in the Lunugamvehera MOH area due to its agricultural setting may have influenced the observed high richness and diversity of anophelines. Similar observations of high *Anopheles* diversity in agricultural landscapes compared with the forest landscapes have been reported in northern Thailand (Overgaard *et al.*, 2003).

Breeding habitats and biting behaviour of *Anopheles*

A total of 18 types of positive breeding habitats were identified in all four study sites. The major breeding habitats of the districts for the *Anopheles* were tanks, canals, ground pools, and lagoons. Different *Anopheles* species were observed to have different breeding habitat preferences. *The An. culicifacies* larvae were recorded from canal margins, stream margins, tank margins, quarry pits, and temporary water collections. The *An. subpictus* larvae were observed in breeding habitats, including in brackish water bodies, such as lagoon margins, marshy lands, ground pools, tank margins, wells, burrow pits, and temporary water collections. The *An. vagus* was found in freshwater bodies such as temporary water collections and canals. The *An. varuna* was recorded from a variety of breeding habitats such as ground pools, wells, ponds, tanks, canals, and stream margins.

A total of 6243 *Anopheles* adult mosquitoes were collected through the CBTC (90.5%, n=5652), CBHC (8.3%, n=523), and HLNC (1.1%, n=68) techniques from the Arabokka, Mirijjawila, and Mattala locations. The primary vector, the *An. culicifacies*, was recorded in at least one adult sampling technique in all three study sites. The *An. subpictus* was the predominant species detected from all adult sampling techniques, including CBTC (82.3%, n=1834), CBHC (15.4%, n=343) and HLNC (2.2%, n=50). THE *An. nigerrimus* was detected in high densities from the CBTC in all three localities. The *An. culicifacies* was abundant in the CBHC technique, implying that the primary vector in the Hambantota district is endophilic nature.

The human biting behaviour of malaria mosquitoes is an important determinant when designing vector control programmes. Host feeding is a complex behaviour and is determined by a combination of both innate host preferences and ecological factors, such as host seeking behaviour (Burkot *et al*., 1988) and host availability, including defensive behaviour (Lyimo *et al*., 2009). To establish which control measures would be most effective in an environment with multiple vectors, species-specific feeding behaviours must be clearly defined (Laurent *et al.*, 2017). In this study, the relative biting preferences of the *Anopheles* mosquitoes and their peak biting times were studied. The highest relative biting preference was shown by the *An. subpictus* (87%) followed by the *An. culicifacies* (11%) and the *An. nigerrimus* (2%) in the Hambantota MOH area. The biting peak of the *An. subpictus* was 1900 to 2100 hours (Figure 1). The biting peak of the *An. culicifacies* was 2000 to 2300 hours, whilst the biting of the *An. nigerrimus* peaked during 2100 to 2200 hours.

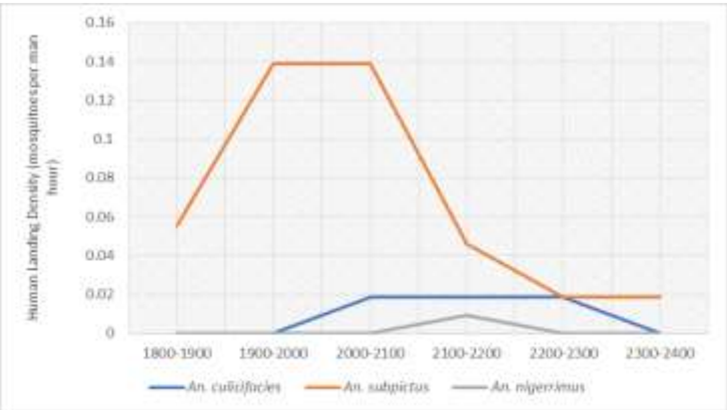

Figure 1. Biting times of *Anopheles* vector mosquitoes in Hambantota MOH area

In the Lunugamvehera MOH, the highest relative biting preference was shown by the *An. culicifacies* (11, 69%) followed by the*An. subpictus* (5, 31%). The biting peak of the *An. subpictus* was 1900 to 2000 hours and the biting peak of the *An. culicifacies* was 2000 to 2100 hours in the Lunugamvehera MOH (Figure 2).

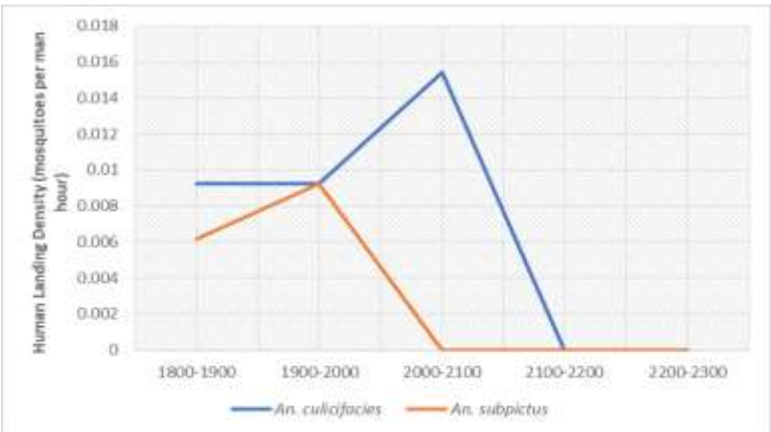

Figure 2. Biting times of the *Anopheles* vector mosquitoes in the Lunugamvehera MOH area

Both MOH areas are highly malaria receptive with a predominance of the human - biting *An. culicifacies* and *An. subpictus,* the primary and secondary vectors of malaria in Sri Lanka. The collections of the *An. culicifacies* and *An. subpictus* peaked around 1900 to 2100. During this period, people are more likely to be still active rather than sleeping and thus, unlikely to be protected under bed nets and LLINs. Hence, it is important to consider other vector control interventions such as IRS when designing vector control programmes in the District of Hambantota.

CONCLUSIONS

The present study provides knowledge on the prevalence of the *Anopheles* mosquitoes in the selected sites in the District of Hambantota and their bionomics during a period of six months. Current knowledge, together with such studies repeated annually, would be useful in designing appropriate vector control measures in the District of Hambantota to ensure the prevention of the reintroduction of malaria in Sri Lanka.

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