Transformation of vector abundance in Mannar District of Sri Lanka Indicating a Potential Entomological Risk for Malaria Transmission; a Comparative Study of the Present and Past

PJ Jude^{1,2}, PADHN Gunathilaka^{1#}, SD Fernando^{3,4}, PH Premarathne⁴, AR Wickramasinghe⁵, SE Priyatharshan⁶, and W Abeywickrama⁴

¹Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka.
 ²Department of Zoology, Faculty of Natural Sciences, Open University, Sri Lanka.
 ³Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka.
 ⁴Department of Para-Clinical Sciences, Faculty of Medicine, Kotelawala Defence University, Ratmalana, Sri Lanka.
 ⁵Department of Public Health, Faculty of Medicine, University of Kelaniya, Sri Lanka.
 ⁶Regional Office, Anti-Malaria Campaign, Mannar District, Sri Lanka.

Abstract

Until recently, malaria was a leading cause of morbidity and mortality among people in Sri Lanka. In 2016, the WHO certified the country as "malaria free". The identification of anopheline mosquitoes is an important aspect in malaria surveillance and control. Therefore, periodical monitoring and updating of vector abundance and trends are of paramount importance to identify the potential risk of disease transmission and to forecast the entomological risk of a disease outbreak. The objective of this study is determine the trends of vector abundance and probable risk for malaria transmission in the Mannar District of Sri Lanka and compare it with past results. Three sampling locations namely; Erukkalampeddi, Pesali and Tharapura, which were surveyed during previous investigations were selected. The larval surveys were conducted from June 2018 to December 2018 on a monthly basis selecting built wells as breeding sites at each selected locality. Collected immature stages were identified to species level using morphological keys. The results of larval surveys carried out during the years 2010-2014 (in the same locations as above) and 1923-1927 (in the Mannar district) was taken for comparison. The present study encountered four species of anophelines (An. culicifacies, An. stephensi, An. subpictus, An. varuna) from larval surveys conducted in built wells (n= 406). An. culicifacies (34.5%; n= 130) was predominant followed by An. stephensi (15.1%; n= 57). However, An. culicifacies or An. stephensi was not recorded during the surveys conducted between 2010-2014 in Mannar District whilst An. subpictus had been recorded as the most abundant (96.2%) species. Earlier during the period between 1923 – 1927, An. culificacies was recorded at the time where the country was severely affected by malaria infection. Thus, there has been a change in the vector composition and abundance observed over a period of eightyear i.e. from 2010 to 2018. The recent study demonstrates that it was mainly the deep built wells which were positive for malaria vector (57.1%; n= 24), water bodies with clear (79.1%; n= 34) and sunlit (82.6%; n= 38) conditions. The presence of larvivorous fish in the vector breeding habitats was not a considerable factor totally to suppress vector breeding since approximately half of the breeding habitats which were positive for vector observed with larvivorous fish (45.5%). The study highlights the importance of continuous entomological monitoring in order to identify alternative strategies along with the potential risk due to alteration of vector breeding sites and abundance.

Keywords: Malaria, Anopheles, vector, breeding

Introduction

Malaria has been a leading cause of morbidity and mortality among people of the Dry Zone of Sri Lanka throughout its known history. Since October 2012, no cases of indigenous malaria have been reported in Sri Lanka (Gunathilaka *et al.*, 2016). In 2016, Sri Lanka was certified by the WHO as a country which eliminated the malaria, as a life-threatening disease (Shretta *et al.*, 2017). However, there are few challenges for sustaining interrupted transmission and preventing reintroduction in Sri Lanka which are more essential to consider in order to maintain the malaria free status in the country. Identification of new vector species, influx of imported cases and shifting of vector species have been identified as some of challenges (Gunathilaka *et al.*, 2019).

The identification of anopheline mosquitoes is an important aspect in malaria surveillance and control strategy throughout the world. Mannar District of Sri Lanka is a one of the malaria endemic areas in past. There was only limited literature available on the abundance of malaria vectors in this District. Detailed entomological investigations have been carried out in Mannar island in 1913 (James and Gunasekara, 1913) and 1927 (Carter, 1927). The most recent detailed study has been published covering the period of 2010-2014 (Gunathilaka *et al.*, 2019; Gunathilaka *et al.*, 2014). The distribution of malaria vectors, more especially their numerical availability with alteration of vector abundance is a subject of considerable importance in connection with the prevalence and probable dissemination of diseases (Gunathilaka *et al.*, 2019). Therefore, periodical monitoring and update of vector abundance and trends is a paramount importance to identify the potential risk of disease transmission and forecast the entomological risk of a disease outbreak. Hence, the objective of the present study is to update the present trend of vector abundance and probable risk for malaria transmission in Mannar District of Sri Lanka as a comparative study with past verses present.

Method

Study area

District of Mannar is located in the Northern Province of Sri Lanka. It has an area of 2,002 km² with 106,235 human population. When considering the climatic and weather condition, this district has 24.6 - 31.5 °C average temperature and 1,051 mm rainfall annually.

Sampling localities

There sampling location namely; Erukkalmpeddi, Pesali and Tharapura, which were covered by previous investigations were selected for the present study in consultation with the entomological field teams attached to the Anti-Malaria Campaign (AMC) in Mannar District.

Entomological surveillance

As built wells have been identified as the conducive breeding habitat for Anopheles in the district, larval surveys were conducted from June 2018 to December 2018 on a monthly basis selecting built wells at each selected locality. A minimum of 10 attempts were made at each breeding habitat. The *Anopheles* mosquito larvae were classified as early instar stage (I and II) or late instar stage (III and IV) Density was expressed as a percentage of specimens of the species in the whole sample (Banaszak and Winiewski, 1999) according to the formula:

$$D = \frac{1}{L} \cdot 100\%$$

Where:

D- Density, I- Number of specimens of each mosquito species, L- Number of all specimens.

Sample Identification

Mosquito larvae were placed individually in a depression microscopic slide with a minimum amount of water and identified under a light microscope (Olympus Optical Co. Ltd., Tokyo) with an objective (x 10). Stages III and IV instar larvae collected from the field and I & II stages reared to IIIrd stage in the field station were identified using standard morphological keys prepared for the Sri Lankan *Anopheles* larvae (Gunathilaka *et al.*, 2014).

Results and discussion

Species composition of anophelines encountered

The present study encountered 4 species of anophelines from larval surveys conducted in built wells (n=406). *An. culicifacies* was predominant followed by *An. stephensi* (Table 1). These two species have not been recorded from the surveys conducted during 2010-2012. The only recorded species in that study were *An. subpictus* (96.2%) and *An. varuna* (0.19%) together with eight other anophelines (*An. nigerriumus, An. vagus, An. pallidus, An. peditaeniatus, An. jamesii, An. pseudojamesi, An. barbirostris and An. barbumbrosus*). However, during 1923 – 1927 period, *An. subpictus, An. barbirostris, An. nigerriumus, An. peditaeniatus* and *An. culificacies* were the recorded species from the District of Mannar at the time where the country was severely suffered from malaria infection. Therefore, there is a clear change in recent past among vector composition and abundance.

The absence of *An. culicifacies* during 2010-2012, at which the malaria elimination program was operated in the country, there had been a lower risk entomologically for malaria transmission as *An. culicifacies* is regarded as the mail vector for malaria transmission in Sri Lanka. However, the record of *An. stephensi* which is considered to be an urban vector for malaria transmission from Mannar District since 2017 (Dharmasiri *et al.*, 2017) can create a greater entomological risk for disease transmission due to influx of imported cases and illegal migration of risk community from neighboring countries mainly from India (Gunathilaka *et al.*, 2019; Gunathilaka *et al.*, 2014).

Table 1. Vector abundance and density of anophelines encountered

Species	Density (%)				
An. culicifacies	34.5 (n= 130)				
An. stephensi	15.1 (n= 57)				
An. subpictus	9.3 (n= 35)				
An. varuna	41.1 (115)				

Habitat characteristics of the breeding site

Overall, the majority of the built wells which were positive for malaria vector breeding were deep water bodies with approximately 6 -10 meters in average. Many of them were clear (79.1%, n=34) in nature with sunlit (82.6%, n=38) condition. According to previous studies conducted in the Eastern part of Sri Lanka has highlighted that the conducive breeding of anophelines including *An. culicifacies* were recorded from built wells which were in used (Gunathilaka *et al.*, 2015; Gunathilaka et al., 2019). However, the present investigation indicates the opposite trend, which the majority of the built wells positive for vector breeding were abandon in nature. This may cause serious impacts as there is no proper follow up or protective measures for these breeding habitats rather than applying larvivorous fish.

Interestingly it was noted that the presence of larvivorous fish in the vector breeding habitats was not a considerable factor to suppress vector breeding since approximately half of the breeding habitats which were positive for vector observed with larvivorous fish (45.5%). This may be due to the fact that as many of the built wells were abandon in nature, growth of some aquatic vegetation, small rooted plants, organic matter (leaf litter) and contaminated non degradable waste material (polythene, plastic, meatal and rubber) have created ideal hidden places for mosquito larvae. Therefore, alternative methods such as demolition of abandon built wells, fill with sand or use of expandable polystyrene breed to cover the water surface by which hinders the direct contact of gravid female mosquitoes with water for oviposition may be recommended.

	Percentage (%) Habitat characteristics (n)									
	Depth		Appearance		Larvivorous		Lighting		Usage	
Locality					fish					
	Deep	Shallow	Clear	Turbid	Yes	No	Sunlit	Shaded	Used	Not
										used
Pesali	28.6	71.4	90.0	10.0	50.0	50.0	80.0	20.0	70.0	30.0
	(2)	(5)	(9)	(1)	(5)	(5)	(8)	(2)	(7)	(3)
Tharapuram	68.0	32.0	75.0	25.0	37.5	62.5	88.5	11.5	19.0	81.0
	(17)	(8)	(18)	(6)	(9)	(15)	(23)	(3)	(4)	(17)
Erukkalampeddi	50.0	50.0	77.8	22.2	60.0	40.0	70.0	30.0	40.0	60.0
	(5)	(5)	(7)	(2)	(6)	(4)	(7)	(3)	(4)	(6)
Overall	57.1	42.9	79.1	20.9	45.5	54.5	82.6	17.4	36.6	63.4
	(24)	(18)	(34)	(9)	(20)	(24)	(38)	(8)	(15)	(26)

Table 2. Characteristics of the breeding sites observed positive for malaria vectors

The present study confirms that there is an obvious change in vector abundance and distribution in the District of Mannar with compared to the most recent detailed literature documented for 2010 -2012 may be due to environmental changes caused by urbanization, resettlements and development projects in the form of increased mosquito breeding habitats. Therefore, continuous entomological monitoring is compulsory to maintain the achievements, which have already been accomplished in malaria controlling in Sri Lanka.

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References

R. Shretta, R. Baral, A. L. V. Avanceña et al., "An investment case to prevent the reintroduction of malaria in Sri Lanka," The American Journal of Tropical Medicine and Hygiene, vol. 96, no. 3, pp. 602–615, 2017.

S. P. James and S. T. Gunasekara, "Report on Malaria at Talaimannar," Sessional Paper, p. 25, 1913.

H. F. Carter, "Report on malaria and anopheline mosquitoes in Ceylon," Ceylon Sessional Papers, Government Press, vol. 148, pp. 6–65, 1927.

Gunathilaka, N., Hapugoda, M., Wickremasinghe, R., & Abeyewickreme, W. (2019). A Comprehensive Analysis on Abundance, Distribution, and Bionomics of Potential Malaria Vectors in Mannar District of Sri Lanka. Malaria Research and Treatment, 2019.

Gunathilaka N., Abeyewickreme, W., Hapugoda, M., Wickremasinhe, R. (2015). Species composition, breeding habitat diversity and habitat characterization of malaria vector breeding habitats in Trincomalee District of Sri Lanka. Bio Med Research International 2015: 1-10.

Gunathilaka N, Denipitiya T, Hapugoda M, Abeyewickreme W, Wickremasinghe R. Determination of demographic, epidemiological, and socio-economic determinants and their potential impact on malaria transmission in Mannar and Trincomalee districts of Sri Lanka. Malaria J. 2016; 15:330.

Gunathilaka N, Fernando T, Hapugoda M, Wickremasinghe R, Abeyewickreme, W. Revised morphological identification key to the larval anopheline (Diptera: Culicidae) of Sri Lanka. Asian Pac J Trop Biomed. 2014, 3(12): 930-935. doi:10.12980/APJTB.4.2014C941.

J. Banaszak and H. Winiewski, "Podstawyekologii," in Foundation of Ecology, p. 630, Wydawnictwo Uczelniane WSP, Bydgoszcz, Poland, 1999.

Gunathilaka, P.A.D.H.N., Fernando, M.A.S.T., Hapugoda, M.D., Wijeyerathne, P., Wickramasinghe, A.R., Abeyewickreme, W. (2014) Entomological investigations on malaria vector studies in earlier conflict areas of Sri Lanka after 30 years. iPURSE International Research Symposium, Peradeniya University of Sri Lanka, 281.

A. G. Gayan Dharmasiri, A. Y. Perera, J. Harishchandra et al., "First record of anopheles stephensi in Sri Lanka: a potential challenge for prevention of malaria reintroduction," *Malaria Journal*, vol. 16, no. 1, p. 326, 2017