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Distribution pattern of *Anopheles stephensi* (Diptera: Culicidae) in north Malabar region of Kerala and its potential role in malaria transmission

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Abstract
Anopheles stephensi is an important vector of urban malaria in the Indian subcontinent. The present study was planned to investigate the prevalence and distribution of this notorious vector in North Malabar region of Kerala. Larval collections were done from various potential breeding habitats, viz., domestic containers, water collection in the buildings under construction, cement tanks, wells, seepage canals, boats, fresh water ponds, streams, swamp, marshes, artificial containers, overhead tanks and ground level water tanks. The study was conducted in five North Kerala districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad from 2019 to 2021. *Anopheles stephensi* was found breeding in 6 breeding habitats viz., buildings under construction, cement tanks, wells, ponds, boats and ground level water tanks. The positive habitats were distributed in urban, suburban and rural areas. For the first time the species was detected from a non-coastal rural area, indicating its invasion from urban centres. Considering the efficiency of *Anopheles stephensi* as a vector of Malaria, constant surveillance and preventive measures are recommended.

Keywords: *Anopheles stephensi*, habitat preference, primary vector, malaria control

Introduction
Geoclimatic peculiarities and other natural habitats in India form a mosquito-genic condition conducive for the transmission of vector borne diseases. Mosquitoes belong to the top order disease vectors, responsible for spreading several diseases such as Malaria, Dengue, Japanese Encephalitis, Chikungunya, Zika, West Nile Virus and Lymphatic Filariasis. Among these Malaria is one of the major public health problems in India [1].
As per the World Malaria Report 2021 there were an estimated 241 million malaria cases and 627 000 malaria deaths worldwide in 2020. This represents about 14 million more cases in 2020 compared to 2019, and 69 000 more deaths. Approximately two thirds of these additional deaths (47000) were linked to disruptions in the provision of malaria prevention, diagnosis and treatment during the covid pandemic [2]. In India National Vector Borne Disease Control Programme reported 186532 cases and 93 deaths in the same period. The contribution of Kerala was 268 cases and one death.
Anopheles stephensi is an important vector of urban Malaria in the Indian subcontinent [3, 4, 5]. In Kerala, this species was first reported from Kochi in 1992. Subsequently, it was reported from Thiruvananthapuram, Kollam, Thrissur, Valancherry (Malappuram), Kasaragod and Thodupuzha (Idukki) [6]. However, there were no systematic study on the prevalence, habitat specificity and distribution of this important vector species in North Malabar. Hence, the present study was carried out in five Northern districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad from 2019 to 2021.

Materials and Methods
Study area
North Malabar refers to the geographic area of southwest India covering mainly six districts of the state Kerala. These districts are enriched with major and minor water bodies which have strong influence on breeding of mosquitoes especially *Anopheles stephensi*. The study was conducted in five North Kerala districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad.

Sample collection and identification
Immature stages of *Anopheles stephensi* were collected from all possible breeding habitats using 300 ml bowls and dippers. The collected larvae and pupae were transported to the laboratory in plastic containers. For rearing, water samples containing larvae/pupae from each site were covered with nylon cloth to avoid the escape of adult mosquitoes. Emerging adults were collected and anesthetized using diethyl ether, and the newly emerged adults were identified using taxonomic keys [7].

Data on Malaria
Data on Malaria were obtained from the daily bulletins published by the Directorate of Health Services, Government of Kerala on their website.

Results and Discussion

Breeding of *Anopheles stephensi*

During the study period 12 types of potential breeding habitats were surveyed and 6 of them were found to have *An. stephensi* breeding. The positive habitats were curing water (water collection in buildings under construction), cement tanks, wells, abandoned boats in fishing harbours, ponds and sump tanks. The positive habitats were detected from four urban, one suburban and two rural areas (Table 1). Among the breeding habitats only ponds were natural habitats. All others were man-made artificial habits. Except for Ayancheri, all other localities from where An. stephensi was collected were coastal areas. Ayancheri was 12 KM away from the coastline. Breeding was encountered in Kannur, Kozhikode and Malappuram districts.

Table 1: Habitat preference of *An. Stephensi*

Breeding habitats surveyed	Localities – Districts	Urban/ suburban/ rural	Breeding status of *An. stephensi*	
			Positive	Negative
Domestic containers				Yes
Building under construction	Ayanchery-Kozhikode	Rural **	Yes	
Cement tanks	Pakkayil-Kozhikode	Urban *	Yes	
Wells	Pakkayil-Kozhikode Kunjippally-Kozhikode	Urban * Suburban *	Yes	
Seepage water				Yes
Canals				Yes
Boats (abandoned in coastal areas)	Chombala, Koyilandy, Puthiyappa, Beypure_-Kozhikode Ponnani- Malappuram	Urban *	Yes	
Ponds	Pattiad-Kozhikode	Urban *	Yes	
Artificial containers				Yes
Over head tanks				Yes
Sump tanks	Muzhappilangad Kannur	Rural *	Yes	
Fresh water streams				Yes

* Coastal ** Non-coastal

Malaria Cases

From January 2020 to December 2021 a total of 134 cases were reported from the five districts (Table 2). Of these 112 were *Plasmodium vivax* (*Pv*), 15were *Plasmodium falciparum* (*Pf)* and 7 were mixed. While 3 cases were indigenously acquired, the remaining 131 cases were imported cases from other states. The indigenous cases were reported from Kasaragod, Kozhikode and Wayanad districts.

Table 2: Malaria cases reported from January 2020- December 2021

Districts	No. of Malaria cases reported	Type of infection			Indigenous	Imported
		Pv	*Pf*	Mix		
Kasaragod	31	26	3	2	1	30
Kannur	24	20	2	2	0	24
Kozhikode	28	23	4	1	1	27
Wayanad	9	9			1	8
Malappuram	42	34	6	2	0	42
Total	134	112	15	7	3	131

Discussion

Malaria is a tropical endemic disease. In India six species of *Anopheles* act as primary vectors. Among them *An. stephensi* is the most important one. Recently, it has been recognized as an invasive species and has been reported from Sri Lanka and Africa which were free of this species [8, 9]. In Kerala, before the eradication of Malaria in 1965 *Anopheles fluviatilis* was the major malaria vector [10]. However, when malaria re-emerged in the state in 1996 and 1998 (Thiruvanathapuram and Kasaragod respectively), the vector was *Anopheles stephensi* [6]. Hence, *Anopheles stephensi* driven malaria is a recent phenomenon in the state. The present study indicated a diverse assemblage of breeding habitats in the study areas. Though *An. stephensi* is primarily an urban vector, its presence was detected from a rural area also. Besides, breeding was encountered in a non-coastal locality. This shows the spread of the vector from coastal urban centres to the surrounding areas. Though the number of indigenous Malaria reported from the study were very few, the number of imported malaria cases is a matter of concern as they can provide source of infection. Additionally, the invasion of the species to rural areas is also worrisome from the point of view of malaria epidemiology. The confluence of vectors and parasites could trigger

new outbreaks in such areas. Hence, it is necessary to plan strategies to monitor the prevalence of this species and its control.

Conclusion

An. Stephensi is a primary vector of Malaria in India and it has started invading countries outside its primary distribution. In Kerala Malaria due to *An. stephensi* is a relatively new phenomenon. The present study detected the breeding *An. stephensi* in six types of habitats. The most significant observation in the study was the detection of the species in a non-coastal rural area, indicating the invasion of the species from urban centres. Considering the high vectorial capacity and invasiveness of the species, active surveillance for the detection and elimination of this species is warranted in the state.

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