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Meteorological Parameters and Mosquito Species Diversity and Abundance along the Arabian Sea Coastline of Alappuzha District, India: A Year-round Study (2017-18)

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Abstract Tropical countries like India has a huge burden of vector-borne diseases, necessitating studies on mosquito demographics for effective control. In the present paper, we summarize the findings of a 12-month entomological survey conducted to determine the diversity of mosquitoes in human settlements located along the Arabian Sea shoreline in Alappuzha district, Kerala, India. Adult mosquitoes were sampled using modified CDC light-traps operated in dusk to dawn operations at ten trapping sites. Captured mosquitoes were transported to laboratory and identified using standard entomological keys. Shannon's diversity and evenness were calculated to evaluate the richness and diversity of mosquito species. A total of 20 species were identified across five genera. *Culex tritaeniorhynchus* is the eudominant species followed by *Culex quinquefasciatus* and *Culex gelidus*. The seasonal variability of *Cx. tritaeniorhynchus* and *Cx. gelidus*, the two principal vectors for West Nile and Japanese Encephalitis viruses, were studied. The present study provided valuable information about the mosquito demographics and seasonal variability of abundance in human settlements along the Arabian Sea shoreline in Alappuzha, India. Considering the vulnerability of the area to vector-borne diseases due to ecology and presence of migratory birds, future studies may be necessitated to determine the association between vector biodiversity and risk of viral disease transmission to humans.

Keywords India; Mosquito; Species diversity; Abundance; Meteorological parameters; Coastline

Background

Over the last decade there had been a dramatic increase in the spread of vector-borne viral diseases (VBD) worldwide (Shope, 1991; Sudeep et al., 2008; Angle et al., 2008; Manimunda et al., 2010; Gubler, 2010; Sudeep et al., 2011; Dash et al., 2013; Kumawat et al., 2014; Bueno-Mari et al., 2015). Apart from distribution and abundance of vectors, vector-host interactions and complicated vector-pathogen-host interactions, the spread of VBDs has also been found to depend on various climatic factors (Epstein, 1998; Rieter, 2001; Gould et al., 2009; Lafferty, 2009; Dash et al., 2013; Roiz et al., 2014). It has been established that climate change is affecting propagation mosquitoes which in turn has driven the spread of mosquito borne diseases (Monaghan et al., 2018). Resurgence of Dengue, Chikungunya and most recently of Zika resulted in considerable human sufferings and economic burden (Dash et al., 2013; Mourya et al., 2016) in developing countries like India. High mosquito abundance has proved to be a prelude to VBD epidemics (Shil et al., 2018 b; Lafferty, 2009). Meteorological factors like rainfall, relative humidity and temperature (including diurnal temperature range, DTR) play important role in the survival and propagation of mosquitoes as well as vector competence to disease like Dengue, Chikungunya, Malaria, Zika, Japanese encephalitis, West Nile, etc. (Dash et al., 2013; Shil et al., 2018a). This necessitates research to understand the role of climate factors in the spread of mosquito borne diseases.

India has a huge burden of Dengue and Chikungunya (Shil et al., 2018b) with more than 6 million affected between 2010 and 2017 (National Vector Borne Disease Control Programme, Government of India). Apart from these, a sporadic outbreak of West Nile has been reported from southern state of Kerala (Balakrishnan et al., 2016). Recent serological survey in the Alappuzha district, Kerala has revealed 21.5% and 15.9% of those surveyed to be positive for West Nile virus and Japanese encephalitis virus respectively (Balakrishnan et al., 2017).

The Kerala state of India has unique geophysical features like the backwaters along the Arabian Sea coast at places, sandy beaches in some others. While the climate is hot and humid, heavy rainfall occurs under the effects of South West (SW) Monsoon as well as North East (NE) monsoon. The hot and humid tropical climate with high green cover presents ideal settings for mosquito habitats (Balasubramanian and Nikhil, 2015). Abundance of mosquitoes, tourism and rich diversity of local water-birds and their interactions with migratory birds make Kerala a potential breeding ground for zoonotic viral diseases. Coastal Kerala remained a good feeding and nursery ground for a variety of heron, egrets and migratory birds, which are amplifying hosts for Japanese encephalitis and West Nile virus. Recently, Kalaiyarasu et al. (2016) has reported serological evidence of widespread Japanese Encephalitis and West Nile virus infections in native domestic ducks in Kerala. However, there are no comprehensive reports on the role of environmental factors and seasonal variability of vector (mosquito) population, which can propagate these diseases to humans. These necessitate research to understand the role of meteorological factors on abundance and diversity of mosquito demographics in the Alappuzha region of Kerala.

In this paper we are presenting the results of a year-round study on the mosquito population in the coastal villages along the Arabian Sea shoreline of Alappuzha district Kerala, India. We also made an attempt to understand the influence of meteorological parameters on mosquito population and seasonal variability. The purpose of the study was to investigate the seasonal variability in abundance of mosquitoes with stress on *Culex* species, the potential vectors for JE and WN viruses.

1 Materials and Methods

1.1 Survey area and climate

Alappuzha is a district of Kerala state, India with the Arabian Sea on the west, a vast network of backwaters, lagoons and fresh water rivers crisscrossing the land. The total geographical area of district is 38,863 km² and population of 21, 27,789 (Census of India, 2011). The district lies between 9 0 5' to 9 0 55' North latitude and 76 0 17' to 76 0 46' East longitude. The district has 82 km stretch of sea shore. Land-use maps indicate large areas of the district being occupied by wetlands or by rice cultivation.

As per the Koppen classification, Kerala experiences Tropical wet type of climate due to its location at the southern tip of the Indian peninsula. While it is warm and humid year round, bulk of the rainfall occurs from June to October under the influence of South West Monsoon and sporadic rainfall from November-January under the influence of North East Monsoon (Koppen; Weather Atlas of India-IMD). In some years early onset of SW monsoon brings pre-monsoon showers in the month of May.

This district was known to be an area endemic for Japanese encephalitis and West Nile virus. The ecology provided a good feeding and nursery ground for a variety of egrets, heron, and migratory birds which are potential. These birds are amplifying host for Japanese encephalitis and West Nile virus.

1.2 Adult mosquito collection and sampling

Mosquitoes were trapped fortnightly from each location using modified CDC light trap, setup in the premises of houses with consent of the house-owner. A total of 10 fixed trapping locations were considered for the study. The traps were operated from dusk (one hour before sunset) to dawn (two hours after sun rise). All traps were suspended at a height of 1~2 m from the ground in the trees in the premises of houses. Collected mosquitoes were transported the laboratory in ice and identified using standard taxonomical keys (Shil et al., 2018a). All the trapping locations were 10~400 m from the shoreline.

1.3 Meteorological data

Meteorological parameters like monthly average maximum and minimum temperature, rainfall and relative humidity for the coastal villages were obtained from the Rice Research Station (RRS), Kerala Agricultural University-Alappuzha, Kerala.

1.4 Virus detection

The method described by Sudeep et al. (2011), was followed for virus detection and isolation. Only *Cx. tritaeniorhynchus* and *Cx. gelidus* mosquitoes were considered for virus detection.

1.5 Mathematical & statistical analyses:

Mathematical and statistical data analyses were performed in MS Excel and R software.

For better estimation of the mosquito species richness diversity and evenness we have calculated the diversity indices as follows:

a) Shannon-Wiener's Diversity index:

Shannon-Weiner's diversity index is based on the uncertainty that an individual taken at random from the dataset is predicted correctly as a certain species. Larger values represent larger uncertainty and greater diversity of species.

$$\text{Eqn (1)} H' = -\sum_{i=1}^N p_i \ln(p_i)$$

Where $p_i = n_i/N$; p_i =proportion of total sample represented by i^{th} species and n_i is the number of samples of the i^{th} species.

The Shannon Wiener evenness is defined as:

$$\text{Eqn (2)} E = \frac{H'}{\ln(S)}$$

Where S is the total number of species in any geographical location (e.g. total number of mosquito species), H' is the Shannon-Wiener's Diversity index. Value lies between 0 and 1. The value $E=1$ indicate complete evenness which signifies that all the species are equally abundant in the area/location. Evenness is a measure of the relative abundance of the different species making up the richness of an area.

b) Simpson's index of diversity:

Simpson's Index of Diversity (1-D), reflects the probability that two individuals taken at random from the dataset are not the same species (i.e., will belong to different species). It is defined as:

$$1-D = 1 - \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$

Where n_i is the number of the i^{th} species and N is the total number of specimens in the studied location.

The Heydemann's classification was used to evaluate the dominance structure (Spellerberg and Fedor, 2013). This classification has five degrees of dominance: Eudominant species-those making up >30% of all specimens caught, dominant (10%~30%), subdominant (5%~10%), rare (1%~5%) and subrare (<1%).

2 Results

2.1 Study area

The Alappuzha district is shown in the maps displayed in Figure 1. The list of ten villages considered as trapping sites are enlisted in Table 1.

2.2 Mosquito demographics

A total of 6741 adult mosquito specimens were subjected to identification using standard entomological keys. A total of 20 species were identified belonging to five genera viz., *Aedes*, *Anopheles*, *Armigeres*, *Culex* and *Mansonia*. Among the genera, *Culex* was the most diverse (9 species) followed by *Anopheles* (4), *Aedes* (3), *Mansonia* (3) and *Armigeres* (1).

Culex mosquitoes constituted 78.3% of the total catch followed by *Mansonia* with 15.7%, *Armigeres* with 4.62%, *Aedes* with 0.52% and *Anopheles* with 0.86%. The top three most abundant species was found to be: *Cx. tritaeniorhynchus* (41.7%), *Cx. quinquefasciatus* (19.7%) and *Cx. gelidus* (11.2%). As per the Heydemann's classification (Weigmann et al., 1973; Spelberg et al., 2013), *Cx. tritaeniorhynchus* is the eudominant species followed by *Cx. quinquefasciatus* and *Cx. gelidus* as the dominant species. The subdominant species is *Ma. annulifera*. Rare species included: *Ar. subalbatus*, *Cx. sitiens*, *Ma. indiana*, and *Ma. uniformis*.

Overall, considering the 12-months data, the Shannon-Wiener's diversity index was found to be $H' = 1.82$ and Shannon evenness, $E = 0.69$. This signified that all the species are not equally abundant in the study area. The Simpson's index of diversity was found to be $(1-D) = 0.761$, which signified that there existed a strong probability that any two samples selected at random will not be the same species. This meant that high diversity exists.

Figure 1 Location of Alappuzha district in Kerala, India

Table 1 Sites for mosquito trapping in the coastal settlements in Alappuzha district

Sl No.	Name of Village/settlement
1	Ambalapuzha
2	Arthunkal
3	Chethy
4	Vandanam
5	Alappuzha town
6	Karoor
7	Punnapra
8	Thakazhy
9	Thottappally
10	Vadackal

2.3 Meteorological factors and mosquito abundance

2.3.1 Total mosquito abundance

Figure 2 shows the variation of mosquito abundance (MA) with maximum and minimum temperatures (MXT and MNT). While MXT varies over a range of 25.5°C to 30.9°C (annual average ~ 27.8°C, the MNT varies between 15.4°C to 19.5°C (annual average ~ 18.3°C). The temperature range is favorable for mosquito propagation and survival through-out the year. The Pearson's correlation coefficient for MA vs MXT was found to be: $r = 0.069$ (95% confidence, $p\text{-value} = 0.0423$) and that for M vs MNT was found to be $r = -0.480$ (95% confidence, $p\text{-value} = 0.689$). This implies very weak association between temperature variations and mosquito abundance over the study period.

Kerala receives most of the rainfall during the SW monsoon season covering June-July-August-September (JJAS) and some during the post monsoon season covering October-November-December (OND), while January-February-March (JFM) is mostly dry. In Figure 3 we compare the mosquito abundance with the monthly rain fall in the study area. A statistical analysis does not establish any proper correlation between total mosquito abundance (MA) and JJAS rainfall. On the other hand, the MA is negatively associated ($r=-0.96$, 95% confidence) with OND rainfall.

Figure 2 Mosquito abundance, Maximum and Minimum temperatures for the area surveyed

Figure 3 Mosquito abundance and rainfall for the area surveyed

2.3.2 Culex mosquitoes

Demographics indicated that the *Culex* mosquitoes were the dominant genera (78.4%) in the study area. In addition, the Arabian Sea shoreline in Kerala has a high percentage of West Nile and Japanese encephalitis positivity in the local bird population 22. Hence, we have made separate detailed analyses for *Cx. tritaeniorhynchus* and *Cx. gelidus* the two main vectors for these viral diseases.

Figure 4 shows the variation of *Cx. tritaeniorhynchus* abundance with maximum and minimum temperatures (MXT and MNT). The temperature range is favorable for propagation and survival of *Cx. tritaeniorhynchus* through-out the year. The Pearson's correlation coefficient for *Cx. tritaeniorhynchus* abundance vs MXT was found to be: $r=0.1862$ (95% confidence, $p\text{-value}=0.562$) and that for *Cx. tritaeniorhynchus* abundance vs MNT was found to be $r=-0.296$ (95% confidence, $p\text{-value}=0.350$). This implies that *Cx. tritaeniorhynchus* mosquito abundance showed very weak positive association with maximum temperature and a weak negative association with minimum temperature during the study period.

Figure 5 compares the abundance of *Cx. tritaeniorhynchus* and monthly rainfall. The Pearson's correlation coefficient, $r=-0.483$ (95% confidence, $p\text{-value}=0.131$) indicated a moderate negative association between *Cx. tritaeniorhynchus* abundance and monthly rainfall.

Figure 4 Variability in the abundance of *Culex tritaeniorhynchus* mosquitoes with temperature

Figure 5 Variability in the abundance of *Culex tritaeniorhynchus* mosquitoes with rainfall

Figure 6 shows the variation of *Cx. gelidus* abundance with maximum and minimum temperatures (MXT and MNT). The temperature range is favorable for propagation and survival of *Cx. gelidus* through-out the year. The Pearson's correlation coefficient for *Cx. gelidus* abundance vs MXT was found to be: $r=-0.236$ (95% confidence, $p\text{-value}=0.460$) and that for *Cx. gelidus* abundance vs MNT was found to be $r=-0.294$ (95% confidence, $p\text{-value}=0.354$). This implies that *Cx. gelidus* abundance showed very weak positive association with maximum temperature and a weak negative association with minimum temperature during the study period.

Figure 7 indicates the abundance of *Cx. gelidus* and monthly rainfall. The Pearson's correlation coefficient, $r=-0.26$ (95% confidence, $p\text{-value}=0.416$) indicated a moderate negative association between *Cx. gelidus* abundance and monthly rainfall.

Figure 6 Variability in the abundance of *Culex gelidus* mosquitoes with temperature

Figure 7 Variability in the abundance of *Culex gelidus* mosquitoes with rainfall

3 Discussions

The purpose of the study was to conduct a survey of the mosquito population in the coastal human settlements along the Arabian Sea shoreline in the Alappuzha district, Kerala, India so as to understand the demographic diversity and seasonal variations. The aim was to detect the presence of *Culex* mosquitoes, the potential vectors for Japanese Encephalitis and West Nile viruses, these viruses having caused outbreaks in the Kerala state. Mosquito trapping was conducted in 10 fixed locations in the human settlements along the coastal villages between 10–400 m of the shoreline. All houses had gardens and trees planted in the premises, with abundance of fruit trees, with or without vegetable patches or flowerbeds. Small ponds, both temporary (Monsoon season) and permanent (year-round) were also located adjacent to some of the houses. Rice fields or cultivated lands were located around 400 m inland from the shoreline (sea beach). All the trapping sites had similar settings. Mosquito collections were performed fortnightly by light traps operated from dusk to dawn operations (one hour before sunset till one hour after sunrise). Mosquito abundance was calculated in terms of counts per trap.

Mosquito population showed a great deal of diversity in terms of species. A total of 20 species across five genera were detected. Simpson's index of diversity was used to measure the heterogeneity of the mosquito demographics in the study area. High value indicated the high level of diversity of mosquito species. The high value of Shannon-Weiner's diversity index indicated greater richness of the mosquito species. The Shannon-Weiner index of Evenness was found to be high at 0.69, but it still indicated that all the species were not equally abundant in the area. This is similar to findings elsewhere in tropical and subtropical settings in Asia (Nikookar et al., 2015). While there were 9 species from the *Culex* genera, 4 from *Anopheles*, 3 each from *Aedes* and *Mansonia*, there was only one species from *Armigeres*. As per the Heydemann's classification criterion for dominance structure, *Cx. tritaeniorhynchus* was eudominant, *Cx. quinquefasciatus* and *Cx. gelidus* were the dominant species. *Ma. annulifera* was subdominant. All the *Aedes* species were classified as sub-rare and so were the three *Anopheles* species.

Overall mosquito abundance showed seasonal variations. While highest abundance was observed in the month of May 2017, the least was observed in March 2018, which is the peak of the dry season. The influence of meteorological parameters on mosquito abundance was also evaluated in terms of time series analyses and Pearson's correlation coefficient was evaluated as a measure of association. In presence of favorable temperature, mosquito abundance was modulated by rainfall with the high rainfall months recording low abundance probably due to loss of habitat (washed out by flooding).

The study also revealed that *Culex* mosquitoes were most abundant in the area. *Culex* mosquitoes accounted for 78.4% of the total population. *Cx. tritaeniorhynchus* was the most abundant species probably because of humid tropical settings and abundant breeding habitat (closeness to paddy fields). The abundance of *Cx. tritaeniorhynchus* was maximum in the pre-monsoon season. During SW monsoon (JJAS), the rainfall was steady and moderate and *Cx. tritaeniorhynchus* abundance was least. However, *Cx. tritaeniorhynchus* abundance increased during DJF due to less rainfall. Similarly, the abundance of *Cx. gelidus* was maximum in December. Two distinct peaks were observed once in May-June and the other in November-December-January. During the high rainfall months JJAS, the abundance was very low.

The abundance of *Culex* mosquitoes is also influenced by habitat availability. During paddy cultivations in the rainy season (JJAS), reduction of breeding sites may have an impact on the abundance. In Alappuzha district farmers produce two crops a year, with sowing taking place between in November (for winter crop) and an additional season in June (monsoon crop). During the post-harvesting periods (March to May/June and September to October/November) the paddy field areas remained unutilized and the adjoining fields were allowed to have free exchange of water or were flooded on purpose to cause decomposition of weeds along with the paddy stubbles, thus, generating manure. The organic and humic substances thus generated offer rich feed for the *Cx. tritaeniorhynchus* larvae. Thus, larval density, as high as ≥ 350 larvae/dip have been recorded (authors institutional

survey records, hitherto unpublished). Before sowing the water was drained off and tractor tillage practiced for preparing the soil for next cultivation. But the usage of these tractors creates new mosquito breeding habitats (small pits made by tractor tyre) and probably increasing the mosquito density. Major mosquito species recorded in these areas were freshwater mosquitoes such as *Cx. tritaeniorhynchus* and *Cx. gelidus*. The abundance of these vector mosquitoes in the presence of suitable virus reservoir host population (pig, birds, etc.) (Khoobdel et al., 2019) increased public health risk for west Nile and Japanese encephalitis viruses in the human settlements along the Arabian Sea shoreline in Alappuzha.

Work done by Sulesco et al. (2013; 2015) highlights the importance of mosquito surveys in European regions with rich vegetation, large resident bird population and frequent migratory birds. The presence of large bird population (viral reservoirs), advent of seasonal migratory birds each year together with human migrations due to increased tourism and economic activities, the Alappuzha district coastline remain venerable to vector-borne viral diseases. Considering the sporadic outbreaks of West Nile and Japanese encephalitis in the past and with the bird population being reservoirs for these pathogens, it is important to study the vector population in the Alappuzha district, especially along the coastline. The fact that *Cx. tritaeniorhynchus* is eudominant and *Cx. gelidus* being the dominant species has potential human health implications in our study area (Bolling et al., 2009; Barker et al., 2010).

Overall, the 12-months study revealed the demographic diversity of mosquito population in the human settlements along the Arabian Sea shoreline in Alappuzha district, Kerala, India. Key findings included: 1) existence of 20 species in the study area, but all species were not equally abundant (high species richness and moderate evenness as estimated by Shannon indices), 2) higher diversity of *Culex* species with higher degree of dominance for three *Culex* species. Also, the mosquito community was composed of a few abundant and a high number of rare species, establishing a clear relationship between Culicidae abundance and environmental parameters. Though *Cx. gelidus* and *Cx. tritaeniorhynchus* were highly prevalent in the area, West Nile and Japanese Encephalitis viruses were not detected from the field-caught mosquito vectors. The study provided valuable information about the mosquito demographics and seasonal variability of abundance in human settlements along the Arabian Sea shoreline in Alappuzha, India. Knowledge of mosquito demographics and seasonal variability is essential for initiating effective control measures. Considering the vulnerability of the area to vector-borne diseases, it is recommended that further studies should be initiated to determine the association between vector biodiversity and risk of viral disease transmission to humans.

Authors' contributions

Both authors planned and designed the study. RB carried out the field work and entomology laboratory work. PS contributed in data management and mathematical analyses. RB and PS participated in interpretations of the results and manuscript writing. All authors read and approved the final manuscript.

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