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Seasonal prevalence and blood meal analysis of filarial vector *Culex quinquefasciatus* in coastal areas of Digha, West Bengal, India

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ABSTRACT

Background & objectives: Filariasis is one of the major vector-borne diseases causing serious health problem in the tropics and subtropics. The coastal areas of Digha are known to be a filariasis prone region of West Bengal, India. The filarial parasite *Wuchereria bancrofti* is transmitted by *Culex quinquefasciatus*, the established filarial vector in West Bengal, India. The present work was aimed to determine the abundance of different mosquito species; and the frequency, distribution and blood meal analysis of *Cx. quinquefasciatus* in coastal areas of Digha.

Methods: During the present study, a total of 11,537 mosquitoes [*Cx. quinquefasciatus, Armigeres subalbatus, Anopheles barbirostris*, *An. annularis*, *An. subpictus*, *An. sundaicus, Aedes albopictus*, and *Cx. vishnui* (group)] were collected by hand collection method from human habitations and cattlesheds of 10 villages of Digha, West Bengal, India. The seasonal prevalence of *Cx. quinquefasciatus* was studied. In each season, blood meals of 300 *Cx. quinquefasciatus* collected from human habitations were analysed during the study period.

Results: Cx. quinquefasciatus was found to be the dominant species (88.44% of the total collection) in the study area. It was most frequently found in and around human habitations than cattlesheds. Total man hour density calculation revealed that this species was most prevalent during the rainy season. Two-way ANOVA revealed that the abundance of *Cx. quinquefasciatus* varied with different seasons. Blood meal analysis showed that the filarial vector preferred human blood than that of other animals.

Interpretation & conclusion: This study suggested *Cx. quinquefasciatus* as the dominant mosquito species in the study area; and the anthropophilic nature of *Cx. quinquefasciatus* might be the reason of increase in the intensity of filarial transmission in coastal areas of Digha.

Key words Blood meal analysis; *Culex quinquefasciatus*; Digha; filariasis; seasonal prevalence

INTRODUCTION

Arthropod-borne infections are a major public health concern, causing considerable morbidity and mortality in humans and livestock throughout the world[1]. Lymphatic filariasis (LF) is one of the several arthropod-borne diseases that cause serious health problem in the affected population. It is an infection of the human lymphatic system, caused by filarial nematodes transmitted by mosquitoes. The disease primarily affects poor populations living in filariasis-endemic areas of the tropics and subtropics. Currently around 120 million people are estimated to be infected with LF in 83 countries, and >1.4 billion are at risk of acquiring infection[2-3]. Approximately, 80% of these people are living in the tropical countries like Bangladesh, Democratic Republic of Congo, Ethiopia, India, Indonesia, Myanmar, Nigeria, Nepal, Philippines and the United Republic of Tanzania, *etc*. Although, designated by the WHO as the world's second leading cause of permanent and long-term disability, the disease is "potentially eradicable" through drug therapy and vector control measures[4]. The disease is classified into two groups, Bancroftian (*Wuchereria bancrofti*) and Malayan (*Brugia malayi* and *B. timori)*; and *Culex quinquefasciatus* is established as the main vector of *W. bancrofti*, the causative agent of LF in India[5]. Lymphatic filariasis has historically been a significant public health problem in the coastal areas of Digha, India. A sizeable proportion of the population of this area is suffering from filariasis. The mean microfilaraemia rate of this area was found to be 9.1%[5]. As the prevalence and transmission of vector-borne pathogens is determined mainly by the distribution and abundance of the primary vector, therefore, it is essential to study the bio-ecology of filarial vector *Cx. quinquefasciatus* in respect to filariasis. The present study has been conducted to determine the abundance of different mosquito species; and the frequency, distribution and blood meal analysis of *Cx. quinquefasciatus* in coastal areas of Digha.

MATERIAL & METHODS

Study area

For the collection of mosquitoes, a total of 100 human habitations and 100 cattlesheds were fixed in 10 villages (10 human habitations and 10 cattlesheds/village), *viz*. Gadadharpur, Padima, Palsandapur, Jatimati, Chanpabani, Bhagibaharampur, Duttapur, Gangadharpur, Gobindabasan and Somaibasan in the coastal areas of Digha, West Bengal (21.68ºN, 87.55ºE), India.

Collection of mosquitoes

Indoor-resting mosquitoes were captured collectively by four insect collectors between 0600 and 0800 hrs for six min from each human habitation and cattleshed of each village, weekly from November 2013 to October 2014, following hand collection method[6-8]. Permissions were sought from inhabitants of the villages to perform collections in their rooms and cattlesheds for the study. During the study period 10 human habitations and 10 cattlesheds were searched weekly. A total of 384 man-hours (32 man-hours/month) were employed in one year.

Blood meal analysis

Blood meals of 900 (300 in each season) *Cx. quinquefasciatus* mosquitoes collected from human habitations were analysed in each season (summer, rainy and winter) of the study period. Mosquitoes were collected in each month throughout the year from November 2013 to October 2014. Blood-fed wild caught mosquitoes were anaesthetised by chloroform soaked cotton. Then the mosquitoes were identified using standard keys[9-13] and sorted. After identification, blood meal of mosquito was detected by precipitin test by adopting the agar-gel diffusion technique, as described by Ouchterlony and Nilson[14]. It involves the diffusion of antigen (extract protein contained in blood from the stomach of mosquito) and antibody (specific to human, cattle, pig and fowl collected from Institute of Serology, Kolkata, India) through agar gel so that precipitin reaction leaves a distinct precipitate band in the gel, which is easily read.

Statistical alnalysis

Two-way ANOVA and Z-test[15] was performed to analyse the data with the help of Microsoft Excel 2007 and SPSS Statistics 17.0 software.

RESULTS

During November 2013 to October 2014, a total of 5721 mosquitoes were collected from human habitations, out of which 5060 (88.44%) were *Cx. quinquefasciatus*. Other species recorded were *Armigeres subalbatus* (2.62%), *Anopheles barbirostris* (1.7%), *An. annularis* (0.59%), *An. subpictus* (0.79%), *An. sundaicus* (2.59%) *Aedes albopictus* (0.84%), and *Cx. vishnui* group (2.43%) (Fig. 1). A total of 5816 mosquitoes were collected from cattlesheds, out of which *Cx. quinquefasciatus* constituted 51.84% (3015). The corresponding figures for other seven species were 28.03, 12.38, 0.86, 1.03, 1.89, and 3.54% respectively (Fig. 1). Thus, in total, 11,537 mosquitoes were collected during this one year study period, comprising of 8075 *Cx. quinquefasciatus* species. The corresponding figures for other seven species were 1780, 817, 84, 105, 258, 73 and 345 respectively. Hence, it can be said that *Cx. quinquefasciatus* was the most dominating species in the study area.

Man hour density (MHD) of *Cx. quinquefasciatus* and other mosquito species are shown in Fig. 2. The average man-hour collection of *Cx. quinquefasciatus* was 13.18 in human habitations while the figure was 7.85 in cattlesheds. Month-wise analysis of MHD revealed that

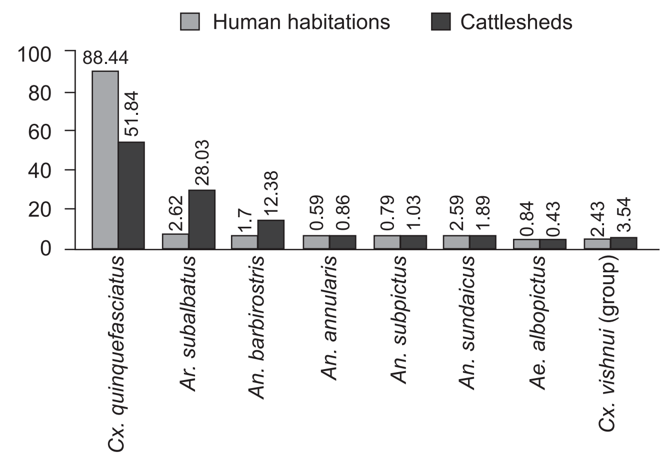

Fig. 1: Percentage of mosquito species collected from the village areas of Digha, West Bengal from November 2013 to October 2014.

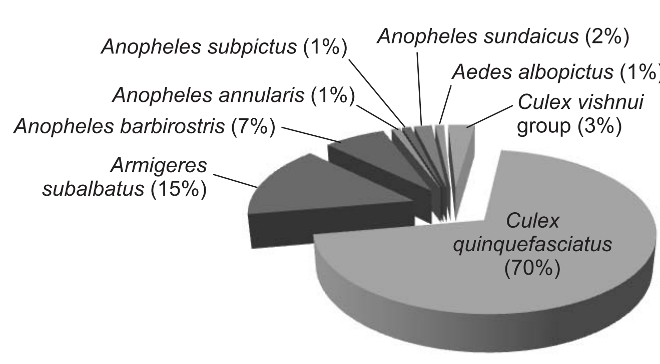

Fig. 2: Overall man-hour density (MHD) of different mosquito species in village areas of Digha, West Bengal from November 2013 to October 2014.

September (20.59) and August (16.66) were the peak density month in human habitations and cattlesheds respectively (Fig. 3). Hence, average per man hour density of *Cx. quinquefasciatus* was very high in the coastal areas of Digha. And out of 5060 *Cx. quinquefasciatus* mosquitoes, 1156, 1466 and 2438 were collected in winter, summer and rainy seasons, respectively from human habitations. From cattlesheds, during winter, summer and rainy season, 465,764 and 1786 *Cx. quinquefasciatus* were collected respectively (Fig. 4). Man hour density in human habitations and cattlesheds in different seasons are depicted in Fig. 5. The per man-hour density was higher during rainy season.

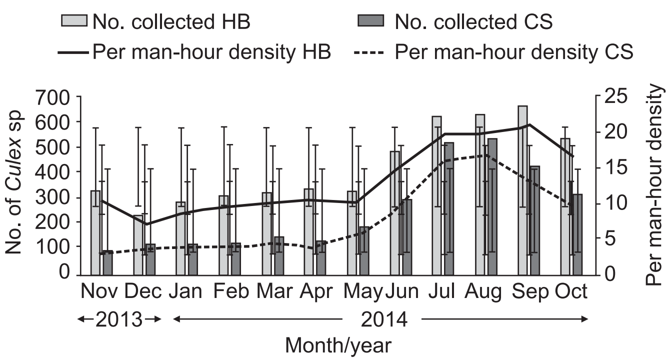

Fig. 3: Total number and per man-hour density of *Culex quinquefasciatus* in human habitations (HB) and cattlesheds (CS) collected from villages of Digha, West Bengal from November 2013 to October 2014.

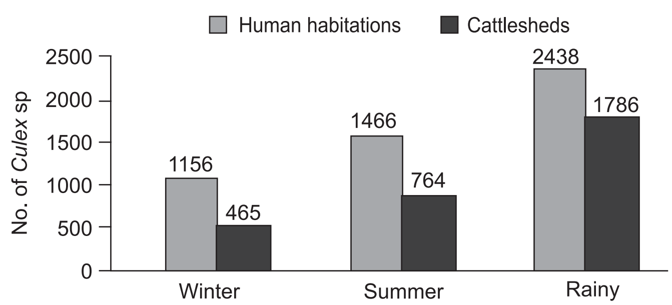

Fig. 4: Total number of *Culex quinquefasciatus* collected from village areas of Digha, West Bengal, in different seasons.

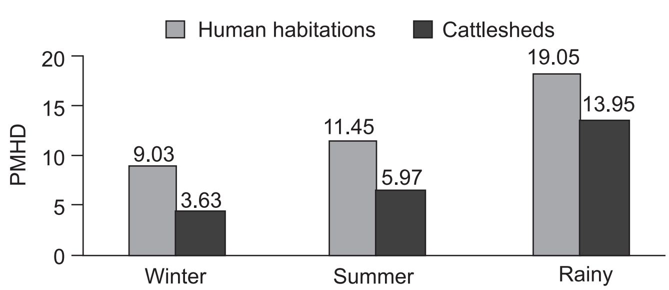

Fig. 5: Per man-hour density (PMHD) of *Culex quinquefasciatus* in village areas of Digha, West Bengal, in different seasons.

The analysis of blood meals of 900 (300 in each season) mosquitoes collected from human habitations, for determining the extent of man-vector contact, revealed anthropophilic index (Human blood index) to be 63.56% in the study area (Table 1). So, man-vector contact was quite high facilitating mosquito–man and man-mosquito transmission of parasite.

Statistical analysis (Z-test) revealed that, *Cx. quinquefasciatus* was significantly higher in rainy season than other seasons in both habitats/sites, *i.e.* human habitations (Z = 2.424) and cattlesheds (Z = 3.6784). During the study it was found that the number of mosquitoes, positive for human blood was not significantly different in different seasons (Z = 0.996). The number of mosquitoes positive for human blood, was significantly higher in rainy season (Z = 6.80) and summer (Z = 4.20), than for pig, poultry birds and cattle. The two-way ANOVA showed that the number of this mosquito significantly varied according to different habitat and as well as different seasons ($p<0.05$) (Fig. 6).

Table 1. Blood meal analysis of *Cx. quinquefasciatus* mosquitoes in winter, summer and rainy seasons from November 2013 to October 2014

Seasons	Blood meal tested	No. and percentage of mosquitoes positive for			
		Human	Cattle	Pig	Fowl
Winter	300	170	110	2	18
Summer	300	168	120	3	9
Rainy	300	234	60	1	5
Total	900	572 (63.56)	290 (32.22)	6 (0.67)	32 (3.55)

Figures in parentheses indicate total percent positive.

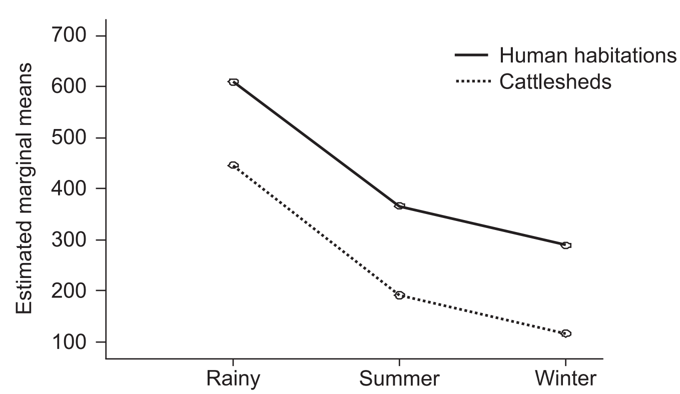

Fig. 6: Profile plot of No. of *Culex quinquefasciatus* in human habitations and cattlesheds during different seasons of the study period.

DISCUSSION

A good number of mosquito species was found prevalent in human habitations and cattlesheds in the study area. Among the mosquito species collected, *Cx. quinquefasciatus, An. sundaicus* and *Ae. albopictus* preferred human habitation than cattleshed as resting site, whereas *Ar. subalbatus*, *An. barbirostris*, *An. annularis*, *An. subpictus* and *Cx. vishnui* (group) preferred cattleshed than human habitation. The cause of such variation in preferring resting sites might be linked to their blood feeding habit (anthropophagic or zoophagic *etc.*).

The number of *Cx. quinquefasciatus* found in human habitation was significantly higher than that of cattlesheds which indicated that this mosquito species preferred human habitations rather than cattlesheds as their resting site. This is an important point in respect to man-vector proximity, leading to disease transmission. The present study depicted that *Cx. quinquefasciatus* was the most dominating house-frequenting mosquito species as also reported earlier by Iyenger[16], Chand *et al*[17], Cader *et al*[18], and Rudra and Chandra[19] in their studies. Probably suitable and varied breeding sites favoured this species to build up such high density. Species composition of mosquitoes in an area has an important/significant role in the filarial transmission dynamics[20]. The filarial vector was found in considerable number in the human habitations (Figs. 1 and 4). Density of filarial vector was quite high in the study areas, which indicated that breeding facilities of the mosquitoes were very more favourable in the study area. Total number of mosquito collected during the study and MHD in the human habitations were higher ($p<0.05$) than those of cattlesheds (Figs. 1 and 2). The average man-hour density of *Cx. quinquefasciatus* was quite high in the study area in comparison to other areas in West Bengal[21–22]. The WHO Expert Committee on filariasis has stressed on this aspect, as human habitations with more filarial vectors are known to be of high epidemiological importance[6]. In the study area the number of *Cx. quinquefasciatus* was very high due to increase in breeding sites during rainy season in drains, ditches, cesspits and cesspools, *etc*. During rainy season more number of temporary breeding places, humid environment and optimum temperature favoured breeding as well as survival rate of this vector species in coastal areas. During summer season large ponds and aquatic marshylands are converted into shallow aquatic bodies which act as breeding ground of these mosquito species. The temperature in summer probably influenced the rapid metamorphosis of this vector species. Similar observation was recorded by De and Chandra[23] in North 24 Parganas, West Bengal. Lower humidity and lower temperature below the optimum limit in winter were not favourable for the breeding of mosquito species. So the lowest prevalence was observed in winter. The present investigation was similar to Burnhes[24], Hayes and Downs[25], and Rudra and Chandra[19] in contrast to the observations of Verma *et al*[26] and Chandra *et al*[27].

Host feeding patterns determine both the frequency of blood feeding by vectors, and the ability of the vector to transmit disease agents. Mosquito feeding patterns vary among different species[28]. Observed differences in the blood feeding pattern may result from seasonal fluctuations in mosquito abundance[29], residual spraying[30] and host availability[29, 31]. By means of screening epidemiological situation of a filarial prone area, more emphasis had been given on the identification of the source of blood taken by female vector mosquito[32]. This reporting is also significant in the study of filariasis. Owing to the discovery of the resting habitats of *Cx. quinquefasciatus*, an opportunity was afforded for large scale analysis of fresh blood meals of captured *Cx. quinquefasciatus*, the findings of which are reported in this study. Blood meal analysis of *Cx. quinquefasciatus* of the present study area revealed 63.56% anthropophilic index and 36.44% zoophilic (cattle, pig and poultry birds) index in human habitations. Anthropophilic index of *Cx. quinquefasciatus* has been worked out by different researchers[33-35]. The present observation showed similarity with the study of Nair and Samanta[33] in Gujarat and Chatterjee and Hati[35] in Kolkata, which authenticated the work.

CONCLUSION

Present investigation gave a clear idea about the host seeking behaviour of *Cx. quinquefasciatus*, with man being the decidedly preferred host; increasing the intensity of filarial transmission in coastal areas of Digha. Higher anthropophilic index might have caused higher man vector contact as well as higher level of disease transmission.

Conflict of interest

We declare no conflict of interest associated with this manuscript.

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