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Studies on breeding habitats and density of postembryonic immature filarial vector in a filarial endemic area

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ABSTRACT

Objective: To obtain a complete and systematic data about the breeding habitats and density of vector immatures (larvae & pupae) in a filarial endemic area. **Methods:** All the possible permanent and temporary water bodies were surveyed systematically. Four hundred samples were taken in each season from each type of habitats. Sampling was done with a 250 mL dipper and immature mosquitoes were identified following standard keys. **Results:** In summer, rainy and winter seasons, overall 49.64%, 44.64%, and 28.57% of the habitats were positive for immature filarial vector (*Culex quinquefasciatus*) respectively; 36.93%, 35.11%, and 21.18% of the samples were positive for immatures respectively and overall per dip densities (PDD) of them were 10.29, 10.18, and 4.40 respectively. In the study year overall PDD were much higher in open cesspits (19.53) and drains (17.24) than in other habitats. Peak PDD of vector immatures in *Dobas* (ditches), open cesspits and drains were in March whereas in paddy fields and temporary water bodies peak PPD were in September. **Conclusion:** *Dobas*, paddy fields, open cesspits, drains, and temporary water bodies were found to be the main breeding sites of filarial vector, *Cx. quinquefasciatus* in different months. Open cesspits and drains were suitable almost throughout the year with peak in summer. *Dobas* also act as a steady breeding site throughout the year, whereas temporary water bodies immerge as strong breeding site during pre-monsoon and monsoon seasons. Information about the breeding habitats will be helpful to formulate a filarial vector control strategy and in turn to control the filarial diseases in the study area.

1. Introduction

The mosquitoes are the vectors of many of the diseases causing major human health problems[1,2]. Besides treatment of the diseases, other approaches for controlling mosquito borne diseases is the interruption of disease transmission by either killing or preventing mosquitoes to bite human beings (by using repellents) or by inducing larval mortality in a large scale at the breeding centers of the vectors. Nowadays mosquito control is largely focused on killing of larva in their habitats using chemical or biological control agents[3-13]. However, complete and systematic information about the breeding habitats of the vector and its density in any area is essential to formulate an effective vector control strategy, which has not been given due importance in many studies. Instead of that, mosquitocides and larvaecides, mostly the chemical ones, are used in non-specific way to all the habitats in any season. In general, the breeding habitats of mosquito and density of their larvae and pupae vary according to the local conditions like climatic factors, physicochemical parameters, presence of predators *etc*[14-17].

Chatterjee *et al* while conducting a survey in metro rail construction sites in Kolkata of West Bengal found that, *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mainly preferred foul water for breeding in winter and post-winter months[18]. A wide variety of sites, mostly characterized by coloured foul water with high nutrient values and low dissolved oxygen, such as pumping and irrigation wells, canals, wastewater treatment ponds, sewage overflows, rain pools, rice paddy fields, fish ponds, septic tanks, drains, cesspools, agricultural trenches, vegetable farms *etc.*, were preferred as breeding habitat by *Cx. quinquefasciatus* in different areas[19-27].

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In India, mosquito control programme is generally linked with sanitation and solid waste disposal, which is carried out by local bodies like municipalities or *panchayats*[28]. Most of them are unable to undertake effective mosquito control due to various technical and operational reasons, which largely increases the mosquito and mosquito born disease problems in the country.

Villages around the foothills of Susunia of Bankura District, West Bengal, India are endemic for bancroftian filariasis[29]. To gather first hand information, all kinds of water bodies in the study area were searched to ascertain the density of immature (larvae and pupae) of *Cx. quinquefasciatus* and its habitats, which may be helpful in formulating vector control strategy.

2. Materials and methods

For a one-year study, 8 villages around the foothills of Susunia, Bankura District, West Bengal, India were selected. From each village permanent common water bodies like 5 fresh water fish ponds, 5 *dobas* (small polluted ponds, may be compared with ditches), 5 wells, 5 paddy fields, 5 open cesspits and 5 drains (stagnant or almost stagnant) were fixed for sampling of breeding sites (Figure 1). Sampling was made using a 250 mL dipper, adopting suggested dipping methods of World Health Organization and Service, with some modification[30,31]. Collection was made in the morning (7:00-10:00 am), during the year 2009-2010.

Figure 1. Different habitats acting as breeding places of *Cx. quinquefasciatus* in the foothills area of Susunia of Bankura District, West Bengal, India. a: *Doba*; b: Well; c: Paddy field; d: Open cesspit; e: Drain; f: Temporary water body (ground rain water).

One year was divided into 3 seasons, namely summer (March-June), rainy (July-October) and winter (November-February). Mosquito breeding sites of each of those 8 villages were searched twice in each season. 5 dips were taken at random on 5 different spots of each selected water body, twice in a season. Four hundred dips were taken from each type of breeding site per season (8 villages×2 times in a season×5 habitats×5 dips).

Random collections of immature larvae and pupae were also made from all sorts of temporary water bodies, created mainly after the rain. For smaller water bodies, where dipping was not possible, contents (water and immature) were collected by siphoning or using dropper and then measured.

The dippers were always immersed slowly in the breeding places at an angle of 45° for the obvious reason of not to disturb the immature vectors. Then the surface water would flow into the cavity of the dipper; care had been taken not to fill the dipper with water completely as the specimens might be washed away. An interval of 2-3 minutes was maintained between subsequent dips, to allow the immature to return to the surface. Collection was avoided immediately after heavy rain for the reason that there was possibility of the immature being washed out from their breeding places. After each dip the immature were transferred from the dipper to the wide mouthed bottle with the help of pipette and dropper. Water from breeding places was taken to fill half of the bottle. The bottles were covered with perforated caps, labelled properly with date at the place of collection. The collecting bottles were then placed in bags and carefully carried to the laboratory without excessive shaking.

In the laboratory, the 3rd and 4th instars larvae were identified under hand-lens or microscope in living condition after keeping in cavity slide. If the larvae were very active during examination they were taken in a tube, a little chloroform vapour was applied into the tube and then it was placed onto the slide. The first and second instars larvae were reared in the laboratory and were identified in the above mentioned process when they attained 3rd or 4th instars. Pupae were allowed to hatch and then the adults were anaesthetised with chloroform and identified under hand-lens or microscope. When any controversy arose, regarding the identity of any larval species it was reared up to adult stage and identified. Identification was done following standard keys[32,33].

When any larva died during transportation, it was placed in carbolic acid for 20 min for dechytinization. Then it was taken in a slide and the extra carbolic acid, which adhered to its body was soaked with the help of a blotting paper. Then it was mounted using Euparal and identified. The number of immature mosquitoes of different species was counted and noted for each day of collection for each breeding place.

Abundance *i.e.*, per dip density (PDD) of immature was determined as the mean frequency of occurrence per 250 mL water. Available data were subjected to statistical analyses using arithmetic mean, standard normal deviate 'Z' and student's '*t*' test[34].

3. Results

Out of 6 types of permanent habitats searched during the study, 5 were recognized as breeding sites of *Cx. quinquefasciatus*. Temporary water bodies were also found to be major breeding sources of the mosquito.

During the study period overall 53.33%, 13.33%, 23.33%, 70.83%, 78.33% and 45.83% of the studied *dobas*, wells, paddy fields, open cesspits, drains, and temporary water bodies were found to be positive for immature vectors (larvae and/or pupae) of *Cx. quinquefasciatus* respectively. No immature stages of the vector mosquito were noticed in the studied Ponds. In summer season 67.50%, 17.50%, 27.50%, 95.00%, 87.50% and 52.50% of *dobas*, wells, paddy fields, open cesspits, drains and temporary water bodies was positive for immature vectors respectively. Whereas in rainy season 52.50%, 10.00%, 42.50%, 47.50%, 75.00% and 85.00% was positive; in winter season 40.00%, 12.50%, 0.00%, 70.00%, 77.50% and 0.00% of those habitats were found to be positive respectively (Table 1).

Overall 24.58%, 6.33%, 12.33%, 62.67%, 71.42% and 40.17% of the samples taken from *dobas*, wells, paddy fields, open cesspits, drains and temporary water bodies were found to be positive for immature vectors of *Cx. quinquefasciatus*. In summer months 34.75%, 7.50%, 13.75%, 86.25%, 75.25% and 41.00% of the samples and in rainy months 24.25%, 5.00%, 23.25%, 36.50%, 77.25 % and 79.50% of the samples taken from *dobas*, wells, paddy fields, open cesspits, drains and temporary water bodies were found to be positive. Whereas in winter months 14.75%, 6.50%, 65.25% and 61.75% of the samples taken from *dobas*, wells, open cesspits, and drains were found to be positive respectively. Paddy fields and temporary water bodies cannot be sampled in winter because water content was either very low or nil (Table 1).

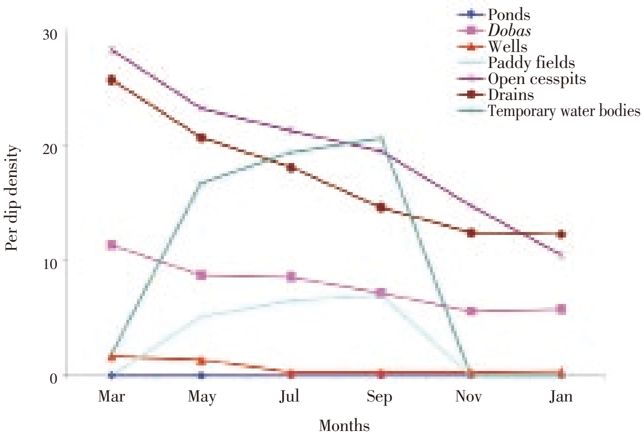

Figure 2. Fluctuation in density of *Cx. quinquefasciatus* immature vectors of different habitats in different months.

Table 1.
Seasonal variation of positive habitats, positive samples and density of the immature of *Cx. quinquefasciatus* in the foothills area of Susunia of Bankura District, West Bengal, India.

Breeding sites	Percent of positive habitats				Percent of positive samples				Per dip density			
	Summer	Rainy	Winter	Overall	Summer	Rainy	Winter	Overall	Summer	Rainy	Winter	Overall
Ponds	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dobas	67.50	52.50	40.00	53.33	34.75	24.25	14.75	24.58	9.99	7.86	5.67	7.84
Wells	17.50	10.00	12.50	13.33	7.50	5.00	6.50	6.33	1.46	0.23	0.26	0.65
Paddy fields	27.50	42.50	0.00	23.33	13.75	23.25	0.00	12.33	2.54	6.67	0.00	3.07
Open cesspits	95.00	47.50	70.00	70.83	86.25	36.50	65.25	62.67	25.72	20.29	12.59	19.53
Drains	87.50	75.00	77.50	78.33	75.25	77.25	61.75	71.42	23.13	16.29	12.31	17.24
Temporary water bodies	52.50	85.00	0.00	45.83	41.00	79.50	0.00	40.17	9.23	19.94	0.00	9.72
Overall	48.93	44.64	28.57	40.71	36.93	35.11	21.18	31.07	10.29	10.18	4.40	8.29

Table 2.
Climatic parameters of the region during the year 2009-2010.

Parameters		Month					
		Mar 2009	May 2009	July 2009	Sept 2009	Nov 2009	Jan 2010
Average temperature (℃)	minimum	19.4	23.7	25.5	25.0	17.9	10.0
	maximum	35.8	36.1	32.4	32.9	30.0	25.8
Average humidity (%)	lowest	17.7	40.0	68.2	63.4	35.0	24.5
	highest	65.2	86.4	93.9	93.6	82.4	80.8
Total rainfall (mm)		30.4	112.6	145.0	70.7	4.0	0.0

During the one-year study period overall PDD of the larvae and pupae of *Cx. quinquefasciatus* was 8.29. The habitats like *dobas*, wells, paddy fields, open cesspits, drains and temporary water bodies were found to be with overall PDD of 7.84, 0.65, 3.07, 19.53, 17.24, and 9.72 respectively during the study. In summer PDD in those habitats were 9.99, 1.46, 2.54, 25.72, 23.13 and 9.23 respectively and in rainy season the corresponding figures were 7.86, 0.23, 6.67, 20.29, 16.29 and 19.94 respectively. In winter PDD in *dobas*, wells, open cesspits and drains were calculated to be 5.67, 0.26, 12.59 and 12.31 respectively. In fresh water fish ponds no immature stages of *Cx. quinquefasciatus* were noticed in all the three seasons and in paddy fields and temporary water bodies during the winter season (Table 1).

Month wise analysis shows that, PDD in open cesspits and drains was higher in the month of March (28.23 and 25.63) and then decreased in the following months (lowest in January, 10.43 and 12.25). PDD in *dobas* maintained almost a steady state in all the months (ranges 5.60 – 8.67) except with little higher value in March (11.31). PDD in paddy fields and temporary water bodies started increasing from the month of May (5.08 and 16.66) and reached the peak in September (6.95 and 20.55) (Figure 2).

According to the data collected from regional meteorological departments, average minimum and maximum temperature varies between 10.0 ℃ to 25.5 ℃ and 25.8 ℃ to 36.1 ℃ respectively in different months. Average lowest and highest humidity varies between 17.7% to 68.2% and 65.2% to 93.9% respectively in different months. Whereas, monthly total rainfall ranges from 0.0 mm to 145.0 mm in different months (Table 2).

4. Discussion

Early studies in the area identified *Wuchereria bancrofti* as the causative filarial parasite and *Cx. quinquefasciatus* as the filarial vector[35]. Overall man hour density, infection rate and infectivity rates were assessed as 10.54, 6.31% and 1.38% respectively. Different indices related to the vector were much higher in summer, which indicates that, summer is the most favorable season for transmission of bancroftian filariasis in the study area[36].

Present study depicts that a good source of suitable water bodies available in the area throughout the year for breeding of the filarial vector *Cx. quinquefasciatus*. *Dobas*, open cesspits, drains and temporary water bodies were the preferred breeding sites of the filarial vector in the study area. In summer and winter months significantly higher percentage of open cesspits and drains act as breeding place of the vector in comparison to all other habitats (P<0.05). Whereas, in the rainy season, drains and temporary water bodies form a significantly higher percentage of breeding places (P<0.05). Overall percentages of habitats positive for vector immature in the study area, in all the three seasons were quite higher[18,19].

Similarly, in summer and winter percent of samples positive for vector immature in open cesspits and drains were significantly higher than that of other habitats (P<0.05) and in rainy season it was significantly higher in drains and temporary water bodies (P<0.05). Overall percentage of positive samples in the area was lower than some other areas[37].

Considering different months and overall, PDD in wells was very negligible, in *dobas* and paddy fields was moderate, whereas PDD in open cesspits, drains, and temporary water bodies were quite higher in that region. Statistically in summer PDD was higher in open cesspits and drains, in rainy season open cesspits, drains, and temporary water bodies and in winter in open cesspits and drains (P<0.05).

During the survey around the Susunia foothills it was found that, in almost every village there are 6–8 large fresh water fish ponds, mostly located outside the villages and they do not work as breeding sites of the filarial vector *Cx. quinquefasciatus*, which is unlike of other areas[27]. *Dobas* are often closely located with human habitats, which are polluted by the use of people for daily work like washing *etc*. and provide suitable site for vector breeding. Local people vigorously use wells and hence they are not established as good breeding sites. Drainage and sanitation in the area are very poorly maintained and almost every house possesses an adjacent open cesspit or stagnant drain to contain waste water, which appears to be the strongest sites for vector breeding. On the other hand, paddy fields and temporary water bodies act as the vector breeding sites during pre-monsoon and monsoon only, nourished by rainwater. Paddy fields were major breeding sites in many parts of the world[19,20,22]. In the study area, temporary water bodies mostly include accumulated ground water, tree/bamboo holes, discarded earthen pots, plastic/tin cans *etc*., which were established breeding sites of *Cx. quinquefasciatus* in some other studies also[21,38]. It is important to note that, paddy fields and other temporary habitats establish as breeding site in succession, with the age of the habitat[14].

Bankura district of West Bengal, India is located in a dry climatic zone, but the present study reveals that, the months of mid-summer to rainy season provide the most suitable climatic condition for mosquito breeding in the area, influenced by favourable temperature and rainfall. In the rainy season temporary breeding sites are increased with rain water but at the same time immature vectors are washed away from some permanent breeding sites. So, from the present study it appears that, open cesspits and drains act as the most suitable breeding site almost throughout the year; while in addition to those sites temporary breeding sites also immerge as strong place for breeding in the pre-monsoon and monsoon season. As *Cx. quinquefasciatus* is an established filarial vector in the study area[35], information on their breeding sites may also be relevant from an epidemiological point of view.

It is known that the most convenient way of controlling mosquitoes is to control them in their breeding habitats. Therefore, in the light of above findings, season wise or rather a month wise vector control strategy is recommended in those habitats. Moreover different control strategy should be tried locally and habitat wise to find an effective and suitable one. Further study is needed to correlate the systematic information about the breeding habitats and density of the vector immature with an effective control strategy.

Conflict of interest statement

We declare that we have no conflict of interest.

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