



Development status of *Wuchereria bancrofti* in experimentally infected *Culex quinquefasciatus* with seasonal fluctuations: A study in slums of Burdwan

Indranil Bhattacharjee¹, Biplab Mandal², Partha Pratim Chakravorty^{3*}

^{1,3} P.G. Department of Zoology, Raja Narendra Lal Khan Women's College (Autonomous) (Natural and Applied Science Research Centre), Paschim Medinipur, West Bengal, India

¹ Department of Zoology, Dr. Bhupendranath Dutta Smriti Mahavidyalaya, Hatgobindapur, West Bengal, India

² Department of Zoology, Vidyasagar University, Paschim Medinipur, West Bengal, India

Abstract

Changing seasons have effect on development of *Wuchereria* larvae. The effect of seasonal fluctuations on the development were assessed by experimental infection to *Culex quinquefasciatus* from a human volunteer (microfilaria (*mf*) carrier). Study indicates that the rainy season provides optimal conditions for transmission of *Wuchereria* in terms of minimum time span for the development of *mf* into infective L₃ larvae without any apparent loss of parasitic load during the process of development. There exists a robust relationship between ingestion of *mf*, production of L₃ and *mf* density in human blood which is a crucial determinant within the transmission dynamics of filaria.

Keywords: *Wuchereria*, development, fluctuation, season

1. Introduction

Lymphatic filariasis (LF) generally attacks lymphatic system and results in chronic illness. 856 million in 52 countries remain threatened by this diseases. DEC in conjunction with albendazole is recommended as treatment against LF [1]. Stability of transmission of *Wuchereria* depends upon many factors [2, 6] as well as variation within the density of *mf* within the blood and parasite [7, 8]. *Culex quinquefasciatus* is taken under consideration as primary vector of *Wuchereria bancrofti* [9, 10]. High humidity and optimum temperature plays important role in survivality of vector mosquito also as development of *Wuchereria* [11]. Microfilariae (*mf*) required a minimum of 16-17 days within the mosquito to succeed within the infective stage [12]. When the temperature was above 37°C and humidity below 65% no transmission was recorded in Khurda district of Orissa [13]. Many existing literatures are there which aren't sufficient to elucidate the effect of seasonal variation on parasitic development inside the vector species [12, 14, 15, 16].

The present study was designed to assess the effect of differences due to the season on the development of *Wuchereria bancrofti* from *mf* to infective stage (L₃ stage) in *Culex quinquefasciatus* in order that proper strategy even be adopted to manage vector population especially season and it's getting to produce cost-effective results around the year on the highest of things operations.

2. Materials and Methods

2.1 Source, maintenance and Identification of experimental mosquito

Adult blood fed mosquitoes were collected from slum (Hatgobindapur (23.25°C N, 87.97°C E), Pandaveswar (23.70°C N, 87.27°C E), Jamuria (23.70°C N, 87.07°C E) and Memari (23.17°C N, 88.10°C E) of Burdwan during the year March 2019 to February 2020. Adult mosquitoes were then introduced in mosquito cage together with a clear

polyesterene 250 ml cup partially filled with distilled water overnight. Female lay egg one by one arranging them into head down array that sticks together to form the egg raft. Larvae were reared in plastic trays (30 cm × 25 cm × 5 cm) and were fed with Brewer yeast, dog biscuits and algae in a ratio of 3:1:1 respectively [17]. The water was changed on alternate day. The last instars larvae on transforming to pupae were manually collected, transferred to a beaker containing tap water and kept inside a mosquito cage for adult emergence. The emerged adults were put in adult-holding cages and fed with 5% sucrose solution. Key provided by Christophers [18], Barraud [19] and Chandra [20] were used to identify mosquito.

2.2 The Experiment

2.1.1 Volunteers selection

Microfilariae carrier volunteer (sex-male, age- above 18, health status- no clinical signs of filariasis, medicines/treatment taken before- no treatment) from slums of Burdwan were selected at random as hosts for blood meal. Written consent was taken from them after describing them the nature of study. Densities of microfilariae in the blood done by the protocol of Chularerk and Desowitz [21]. At the end of the study they were treated by recommended dose of DEC by WHO [22].

2.2.2 Infection of mosquito

Laboratory reared adult female mosquito (nearly 100) of day 4 age were kept separately in mosquito cage and subjected to starvation for one full day. One hand of human volunteer carrier of *mf* (*Wuchereria bancrofti*) was inserted into the mosquito cage at 1900 hour and allowed the mosquito to imbibe blood. Within one hour interval, nearly about 70% mosquitoes were found to be blood fed. Then the hand of volunteer was withdrawn and glucose solution (5%) was supplied in soaked cotton in the cage.

2.2.3 Volume of the blood ingested by mosquito

The amount of ingested blood was determined by weighing 25 unfed female mosquito before the blood meal and 25 female immediately after the blood meal. The volume of the blood consumed was estimated by dividing the weight differences by 1055 mg/ml (approx. density of human blood).

2.2.4 Number of ingested microfilariae

The expected uptake of *mf* was calculated based on Bryan and Southgate [23]. By dividing the observed *mf* intake by the expected *mf* ingested we estimate the concentration factor. Number of *mf* ingested by mosquito was calculated by multiplying the microfilariaemia of each volunteer by the mean volume of ingested blood. The actual number of ingested *mf* were calculated by dissecting out the gut of the insect. Adult mosquitoes were anesthetized in a test tube by applying few drops of chloroform in a cotton which is used to plug the test tube. Within few minutes mosquitoes were anesthetized. They were placed in a clean glass slide. Legs and wings were removed. Smear was made of the contained blood, fixed in methanol, stained with Giemsa, and the number of ingested *mf* was determined.

2.2.5 Collection of blood from human volunteers to measure mf density

Blood from veins of volunteers were collected using vacuntainers containing EDTA (1 mg/ml) as an anticoagulant. *Mf* density was estimated by counting the

number in 60 mm³ of blood (*mf*/ml).

2.2.6 Development of Wuchereria bancrofti larvae in vector mosquito and collection of meteorological data

Ten mosquitoes were dissected on day 0, 3, 6, 9, 12, 15, 18 and 21 after each blood meal. The head, thorax and abdomen were examined to find whether it was infected by *Wuchereria* larvae. In case of infected mosquitoes, the number of *mf*, 1st, 2nd or 3rd stage larvae were counted in the locations (head, thorax, and abdomen). The experiments were repeated thrice (n=3) in three seasons in a year (summer, rainy and winter). In rainy and summer the experiments were continued up to 15th day of blood meal but in case of winter season the experiments were continued up to 21st day after blood meal. We have also collected data from meteorological department regarding maximum and minimum temperature, rainfall and humidity.

2.2.7 Statistical Analysis

Statistical analysis was done using Z test in order to test the significance difference between average load of *mf*/mosquito on day 0 and average load of L₃/ mosquito on day 15 for summer, rainy and winter season in four different slums of Burdwan under study.

3. Results

3.1 Volume of ingested blood

The mean volume of ingested blood was 4.24±0.53 ml (Table 1).

Table 1: Estimated weight and volume of ingested blood by female *Culex quinquefasciatus*

| Mean weight of mosquitoes (mg) | | Mean weight of blood ingested by mosquitoes (mg) | Mean volume of blood ingested by mosquitoes (ml) |
|--------------------------------|-----------|--|--|
| Unfed | Blood fed | | |
| 1.67±0.16 | 6.15±0.52 | 4.47±0.56 | 4.24±0.53 |

3.2 Number of ingested mf

Mean no. *mf*/infected mosquito varies from 5.50 to 8.00 and

the concentration factor ranged from 1.16 to 1.89 (Table 2).

Table 2: Uptake and concentration of *Wuchereria bancrofti* by *Culex quinquefasciatus* immediately after feeding on blood meal with different microfilarial densities

| <i>mf</i> density in blood meal (<i>mf</i> /ml) | No. of dissected mosquito | Mean no. <i>mf</i> /infected mosquito | No. of <i>mf</i> expected | CF (Concentration Factor) |
|--|---------------------------|---------------------------------------|---------------------------|---------------------------|
| 800 | 20 | 6.43 | 3.39 | 1.89 |
| 1000 | 22 | 5.50 | 4.24 | 1.29 |
| 1250 | 20 | 6.20 | 5.30 | 1.16 |
| 1500 | 21 | 8.00 | 6.36 | 1.25 |

3.3 Measurement of mf density in human blood

The *mf* density ranges between 800-1500 *mf*/ml (Table 2)

examined in three sets of experiments during rainy, summer and winter season (as developmental period is delayed) respectively. Total number of *Wuchereria* larvae obtained in three sets of experiments in three different seasons is presented in Table 3.

3.4 Development of Wuchereria bancrofti larvae in vector mosquito

Altogether 180, 180 and 240 mosquitoes were dissected and

Table 3: Total number of *Wuchereria* larvae obtained in three sets of experiments for each season and four slums

| Name of Slums | Dissected on day | Total number of <i>Wuchereria</i> larvae detected | | | | | | | | | | | | | | | | | |
|---------------|------------------|---|-----|-----|--------|-----|-----|-----------------------|-----|---|-----------------------|---|---|-----------------------|---|---|-----------|---|---|
| | | <i>mf</i> | | | | | | 1 st stage | | | 2 nd stage | | | 3 rd stage | | | | | |
| | | Abdomen | | | Thorax | | | Thorax | | | Thorax | | | Thorax | | | Proboscis | | |
| | | S | R | W | S | R | W | S | R | W | S | R | W | S | R | W | S | R | W |
| H | 0 | 190 | 160 | 169 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P | | 220 | 180 | 190 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| J | | 205 | 170 | 192 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M | | 170 | 150 | 156 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H | 3 | 30* | - | 42* | 25* | 158 | 101 | 89 | 154 | - | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | | | | |
|---|------------------------|------|------|------|-----|-----|-----|-----|-----|------|------|-----|------|------|-----|----|------|------|------|---|
| P | | 40* | - | 64* | 31* | 193 | 120 | 115 | 193 | - | - | - | - | - | - | - | - | - | - | |
| J | | 35* | - | 49* | 29* | 171 | 111 | 103 | 177 | - | - | - | - | - | - | - | - | - | - | |
| M | | 20* | - | 30* | 10* | 145 | 93 | 76 | 143 | - | - | - | - | - | - | - | - | - | - | |
| H | 6 | - | - | - | - | - | - | 32* | - | 89 | 63 | 159 | - | - | - | - | - | - | - | |
| P | | - | - | - | - | - | - | 40* | - | 110 | 101 | 197 | - | - | - | - | - | - | - | |
| J | | - | - | - | - | - | - | - | 38* | - | 101 | 86 | 183 | - | - | - | - | - | - | |
| M | | - | - | - | - | - | - | - | 16* | - | 76 | 54 | 149 | - | - | - | - | - | - | |
| H | 9 | - | - | - | - | - | - | - | 22 | 13** | - | 52 | 61 | 131 | - | - | - | 22 | - | |
| P | | - | - | - | - | - | - | - | 40 | 20** | - | 90 | 90 | 153 | - | - | - | 42 | - | |
| J | | - | - | - | - | - | - | - | - | 35 | 18** | - | 80 | 81 | 140 | - | - | - | 35 | - |
| M | | - | - | - | - | - | - | - | - | 21 | 9** | - | 46 | 43 | 89 | - | - | - | 11 | - |
| H | 12 | - | - | - | - | - | - | - | - | - | - | 57 | 14** | - | - | - | 51 | 151 | - | |
| P | | - | - | - | - | - | - | - | - | - | - | 95 | 23** | - | - | - | 72 | 180 | - | |
| J | | - | - | - | - | - | - | - | - | - | - | - | 86 | 18** | - | - | - | 62 | 165 | - |
| M | | - | - | - | - | - | - | - | - | - | - | - | 51 | 9** | - | - | - | 40 | 131 | - |
| H | 15 | - | - | - | - | - | - | - | - | - | - | 9 | - | - | - | 66 | 40 | 161 | - | |
| P | | - | - | - | - | - | - | - | - | - | - | 12 | - | - | - | 79 | 65 | 190 | - | |
| J | | - | - | - | - | - | - | - | - | - | - | - | 8 | - | - | - | 61 | 56 | 171 | - |
| M | | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | 49 | 31 | 139 | - |
| H | 18 | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 6 | NA | NA | 31 | |
| P | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 10 | NA | NA | 62 | |
| J | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 9 | NA | NA | 50 | |
| M | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 2 | NA | NA | 31 | |
| H | 21 | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 29 | |
| P | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 52 | |
| J | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 36 | |
| M | | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | - | NA | NA | 11 | |
| H | Average load/ Mosquito | 6.33 | 5.33 | 5.63 | | | | | | | | | | | | | 1.33 | 5.36 | 0.96 | |
| P | | 7.33 | 6.00 | 6.33 | | | | | | | | | | | | | 2.16 | 6.33 | 1.73 | |
| J | | 6.83 | 5.66 | 6.40 | | | | | | | | | | | | | 1.86 | 5.70 | 1.20 | |
| M | | 5.66 | 5.00 | 5.20 | | | | | | | | | | | | | 1.03 | 4.63 | 0.36 | |

Number of mosquito dissected on each day=10 and three replicates (10 ×3 =30), S=summer, R= Rainy, W=winter; H=Hatgobindapur, P=Pandaveswar, J=Jamuria, M=Memari*= Degenerating, **=Deformed or Degenerating

Table 4: Z test of four selected slums of Burdwan district in three different seasons (summer, rainy, winter)

| Slums | Statistical parameters | Seasons | | |
|---------------|------------------------|---------|---------|---------|
| | | Summer | Rainy | Winter |
| Hatgobindapur | Difference | 139 | 138 | 138 |
| | Z observed | 37.60 | 46.87 | 55.57 |
| | Z critical value | 1.96 | 1.96 | 1.96 |
| | P value (Two tailed) | <0.0001 | <0.0001 | <0.0001 |
| | Alpha | 0.05 | 0.05 | 0.05 |
| Pandaveswar | Difference | 148 | 138 | 128 |
| | Z observed | 93.60 | 27.23 | 48.37 |
| | Z critical value | 1.96 | 1.96 | 1.96 |
| | P value (Two tailed) | <0.0001 | <0.0001 | <0.0001 |
| | Alpha | 0.05 | 0.05 | 0.05 |
| Jamuria | Difference | 143 | 135 | 142 |
| | Z observed | 42.16 | 41.66 | 21.86 |
| | Z critical value | 1.96 | 1.96 | 1.96 |
| | P value (Two tailed) | <0.0001 | <0.0001 | <0.0001 |
| | Alpha | 0.05 | 0.05 | 0.05 |
| Memari | Difference | 130 | 139 | 125 |
| | Z observed | 37.27 | 152.26 | 24.13 |
| | Z critical value | 1.96 | 1.96 | 1.96 |
| | P value (Two tailed) | <0.0001 | <0.0001 | <0.0001 |
| | Alpha | 0.05 | 0.05 | 0.05 |

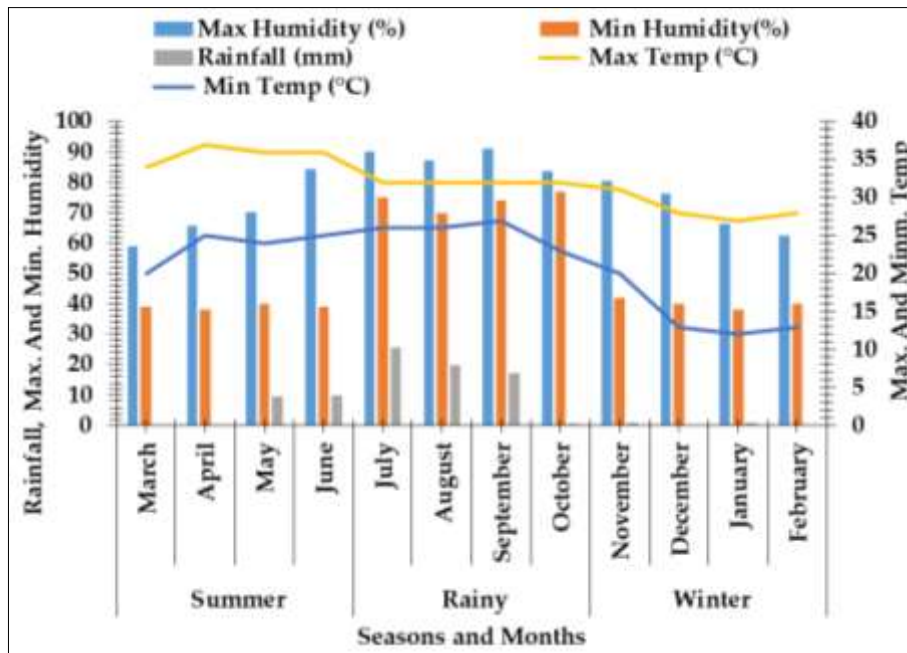


Fig 1: Maximum and minimum temperature, rainfall, maximum and minimum humidity (March 2019 to February 2020)

3.5 Meteorological data

Maximum and minimum temperature, rainfall, max. And min. humidity (March 2019 to February 2020) collected is plotted in Figure 1.

3.6 Statistical Analysis

The results of statistical analysis of four slums is presented in Table 4. In all cases (in all seasons and slums) the P value is lower than than the significant level (alpha level=0.05), therefore, we should safely reject the null hypothesis, and accept alternative hypothesis (H_0 =the difference between the mean is 0; H_a = the difference between the mean is different from 0). It indicates that significant difference existed between the average load of *mf*/mosquito on day 0 and average load of L_3 /mosquito on day 15.

4. Discussions

From past it has been recognized that there are many intrinsic difference in the dynamic relationship between *W. bancrofti* and its vectors necessitates the performance of detailed studies in each endemic area [24, 27]. Our data confirm the positive correlation between *mf* density in the donor blood and the number of *mf* ingested by mosquitoes that has been described by others [28, 25, 23, 29, 30].

During rainy season, *mf* of *Wuchereria bancrofti* required minimum time (9 days) to attain the 3rd infective stage larvae. A certain number of 3rd stage larvae also migrated from thorax to proboscis within 9 days. No deformed or degenerating larvae was detected in the dissected mosquitoes during rainy season.

During summer, *mf* arrived at infective stage within 9 days but none of them migrated to the proboscis of the vectors. Besides, a certain percentages of the larvae were found either deformed or degenerated at different stages of development due to high temperature and less humidity [31].

During winter, *mf* reached the infective stage on day 15 of development migrated to proboscis on day 18. The process of development was delayed due to extremely low temperature and humidity. It was also noted that all the *mf* failed to escape from the midgut of the mosquitoes due to

low temperature as also found by Hu [32] and Chandra [33]. Though no degenerating 3rd stage larvae was observed in the thorax of the mosquitoes, it can be assumed that from the results that all the 3rd stage larvae could not pass from the thorax to proboscis probably due to effect of weather.

In the rainy season, *mf* of *W. bancrofti* reached the proboscis of the vector after developing into 3rd stage very rapidly and average load of the parasites remained more or less unchanged during the course of development in the vector population.

During summer and winter, parasitic development was slow and the average load of parasites in the vector population gradually or sharply decreased with the advancement of parasitic development. It indicates that temperature and humidity are two significant limiting factors involved in filarial transmission. The rainy season is considered to be the high time for transmission of the disease as this season provides optimum conditions to reach the Vector Efficiency Index (VEI) to its peak (which is based on parasitic development, proper nursing and low parasitic damage or death). Therefore personal protection from mosquito bite specifically during rainy season should be an essential and effective step in any filariasis control programme.

5. Conflict of Interest

We declare that we have no conflict of interest

6. Ethical approval

This article is not under consideration or published elsewhere. Ethical clearance for the study was obtained from IAEC, Approval No. 23/IAEC (06)/RNLKWC/2020, dated 08.02.2020.

7. Acknowledgment

Authors are grateful to Principal, PG Department of Zoology, Raja Narendra Lal Khan Women's College (Autonomous) Natural and Applied Science Research Centre, Paschim Medinipore, West Bengal, India for the facilities provided and acknowledge the support to IB to carry out the paper as a part of the thesis under Vidyasagar

University, Raja Narendra Lal Khan Women's College (Autonomous).

8. Authors Contribution:

IB- Data curation, Writing Original Draft, Statistical analysis

BM- Reviewing, Editing

PPC- Designing, Monitoring, Reviewing, Communication

9. References

1. Yahathugoda TC, Weerasooriya MV, Sunahara T, Kimura E, Samarawickrema WA, Itoh M, *et al.* Rapid assessment procedures to detect hidden endemic foci in areas not subjected to mass drug administration in Sri Lanka. *Parasitol Int.* 2014; 63:87-93.
2. Macdonald G. *The Epidemiology and Control of Malaria.* Oxford University Press, London. 1957.
3. Garret-Jones C. Prognosis for the interruption of malaria transmission through assessment of the mosquito vectorial capacity. *Nature.* 1964; 204:1173-1175.
4. Rochet MJ. A simple deterministic model for Bancroftian filariasis transmission dynamics. *Tropical Medicine and Parasitology.* 1990; 41:225-233.
5. Anderson RM, May RM. *Infectious Diseases of Humans. Dynamics and Control.* Oxford University Press, Oxford, 1991.
6. Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, *et al.* The LYMF'HASIM simulation program for modelling lymphatic filariasis and its control. *Methods of Information in Medicine.* 1998; 37:97-108.
7. Southgate BA, Bryan JH. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. Facilitation, limitation and proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg.* 1992; 86:523-530.
8. Failloux AB, Raymond M, Ung A, Glaziou P, Martin PMV, Pasteur N, *et al.* Variation in the vector competence of *Aedes aegypti polynesiensis* for *Wuchereria bancrofti*. *Parasitology.* 1995; 111:19-29.
9. White GB. Lymphatic filariasis, p. 23-34. In *Geographical Distribution of Arthropod-borne Diseases and their Principal Vectors*, World Health Organization, Vector Biology and Control Division, 1989. WHO/VBC/89.967.
10. World Health Organization. *Lymphatic Filariasis: the Disease and its Control*, Fifth report of the WHO Expert committee on filariasis (WHO Technical Reports Series.; n. 821), WHO Geneva, 1992, 75p.
11. Nelson CG. Factors influencing the development and behaviour of filarial nematodes in their arthropodan hosts. Second symposium of the British Society for Parasitology on Host Parasite Relationships in Invertebrate Hosts. Edited by Angela ER. Taylor. Blackwell Scientific Publications, Oxford, 1965, 74.
12. Bancroft TL. *Journal Proceedings Royal Society of New South Wales*, 1899, 82:62.
13. Dash AP, Mahapatra N, Hazra RK, Acharya AS. Transmission dynamics of filariasis in Khurda district of Orissa, India. *Southeast J Trop Med Public Health.* 1998; 29:20-25.
14. Low GG. A recent observation on filaria nocturna in *Culex*: Probable mode of infection of man. *British. Med. J.* 1900, 1:1456.
15. James SP. On the metamorphosis of the filaria *Sanguinis hominis* in mosquito, 1900, 11:533.
16. Rao SS, Iyenger MOT. Experimental infection of some Indian mosquitoes with *Wuchereria bancrofti*. *Indian J Med Res*, 1932, 20:25.
17. Kamaraj C, Bagavan A, Elango G, Zahir AA, Rajakuma G, Marimuthu S. *et al.* Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. *Indian Journal Med Res.* 2011; 134(1):101-106.
18. Christophers SR. *The Fauna of British India, including Ceylon and Burma, Diptera Vol-V*, London: Taylor and Francis, 1933.
19. Barraud PJ. *The Fauna of British India, including Ceylon and Burma, Diptera Vol-IV.* London: Taylor and Francis, 1934.
20. Chandra G. *Mosquito.* Kolkata: Sribhumi Publishing Company, 2000.
21. Chularerk P, Desowitz RS. A simplified membrane filtration technique for the diagnosis of microfilaremia. *J Parasitol.* 1970; 56:623-624.
22. World Health Organization. *Control of Lymphatic Filariasis: A Manual for Health Personnel*, WHO, Geneva, 1987, 89 pp.
23. Bryan JH, Southgate BA. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes Uptake of microfilariae. *Trans R Soc Trop Med Hyg.* 1988; 82:128-137.
24. Samarawickrema WA, Laurence BR. Loss of filarial larvae in a natural mosquito population. *Ann Trop Med Parasitol.* 1978; 72:561-566.
25. McGreevy PB, Kolstrup N, Tao J, McGreevy MM, Marshall TF de C. Ingestion and development of *Wuchereria bancrofti* in *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. *Trans R Soc Trop Med Hyg.* 1982; 76:288-296.
26. Lowichik A, Lowrie RC. Uptake and development of *Wuchereria bancrofti* in *Aedes aegypti* and Haitian *Culex quinquefasciatus* that were fed on a monkey with low-density microfilaremia. *Trop Med Parasitol.* 1988; 39:227-229.
27. Brito AC, Williams P, Fontes G, Rocha EMM. A comparison of two Brazilian populations of *Culex quinquefasciatus* (Say 1823) from endemic and nonendemic areas to infection with *Wuchereria bancrofti*. *Mem Inst Oswaldo Cruz.* 1997; 92:33-36.
28. Obiamiwe BA. Relationship between microfilarial density, the number of microfilariae ingested by mosquitoes and the proportion of mosquitoes with larvae. *Ann Trop Med Parasitol.* 1977; 71:491-500.
29. Janousek TE, Lowrie RC. Vector competence of *Culex quinquefasciatus* (Haitian strain) following infection with *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg.* 1989; 83:679-680.
30. Jayasekera N, Kalpage KSP, De Silva CSS. The significance of low density microfilaremia in the transmission of *Wuchereria bancrofti* by *Culex quinquefasciatus* Say in Sri Lanka. *Trans R Soc Trop Med Hyg.* 1991; 85:250-254.
31. Rozeboom LE, Bhattacharya NC, Gilotra SK. Observations on the transmission of filariasis in urban

- Calcutta. American J of Epidemiology. 1968; 87:616-632.
32. Hu SMK. Observation on the development of filarial larvae during the winter season in Sanghai region. Am. J Hyg, 1939, 29:67.
 33. Chandra G, Chatterjee SN, Banerjee BD, Majumdar G. Effect of seasonal variations on the development of *Wuchereria* larvae in *Culex quinquefasciatus*. J Basic & Applied Biomedicine. 1997; 5(3):21-24.