Microfilarial periodicity of *Wuchereria bancrofti* in Assam, Northeast India


Regional Medical Research Centre (ICMR), Northeastern Region, Dibrugarh, India

**ABSTRACT**

Background & objectives: *Wuchereria bancrofti* has shown nocturnal periodicity in India and other endemic countries of the world except Pacific regions where non-periodic or diurnal sub-periodic forms have been reported. Presence of sub-periodic form of *W. bancrofti* in Andaman and Nicobar Islands, India and a case report of sub-periodic form of *W. bancrofti* from Mysore, India provide basis for exploring the periodicity pattern of microfilaria of *W. bancrofti* prevalent in Assam, Northeastern region of India. State of Assam has unique geographical location as its Northeastern region shares international boundaries with Nepal, China, Bhutan, Myanmar and Bangladesh. Evolutionary association of *W. bancrofti* found in Assam is not known and possibility of its link with *W. bancrofti* form, prevalent in neighbouring countries may not be ruled out. Hence, this study was undertaken to know the microfilarial periodicity of *W. bancrofti* in Assam.

Methods: Ten microfilaria positive adult male individuals having moderate to high microfilaraemia were selected. Informed written consent from each participant was obtained. The presence of microfilaria was observed at two hourly intervals over a period of 24 h using 50 μl of finger prick peripheral blood samples. Peripheral blood smears were processed, stained and examined under microscope and microfilaria counts were recorded.

Results: Data collected were calculated and analyzed using modified statistical method, and the periodicity curve was prepared. Typical nocturnal periodicity was observed at a peak time of 0003 hrs with a periodicity index of 136.2.

Interpretation & conclusion: Analysis of the data revealed nocturnal periodicity of the *W. bancrofti* prevalent in the Assam with peak periodicity about one hour ahead of the other states in India. Findings will be helpful in evaluation and monitoring of ongoing MDA programme for elimination of LF in Assam.

Key words Assam; circadian cycle; lymphatic filariasis; microfilarial periodicity; nocturnal periodicity; *Wuchereria bancrofti*

INTRODUCTION

Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is a neglected tropical disease and poses serious public health problem, affecting 120 million people living in 73 countries of the world1. One-third of the world’s population infected with LF live in India and over 18 Indian states and the union territories are endemic for LF2-3. Approximately, 420 million people reside in endemic areas and 48.11 million are infected2. Bancroftian filariasis caused by *W. bancrofti* accounts for 95% of the total lymphatic filariasis cases in India4. The state of Assam has a large population of tea growing community especially in upper regions of Assam, where a large number of tea gardens are present. High prevalence of LF was recorded from various tea gardens of Assam in earlier studies5-9. The microfilariae of *W. bancrofti* may be found in the blood at all times, but its concentration in the peripheral blood of the infected humans follow a circadian periodic cycle which appears to be synchronized with the biting habits of the mosquito vectors10-12.

Nocturnal periodic form of *W. bancrofti* is widely distributed in tropical and subtropical regions like Africa, Asia and Latin America while non-periodic or diurnal sub-periodic form is prevalent in the Islands of the South Pacific regions where maximum densities of microfilaria (mf) count is observed around 1630 hrs; and the distribution of sub-periodic form of *W. bancrofti* is limited to western Thailand where peak mf density in the peripheral human blood is observed at 2030 hrs13-15. In India also, sub-periodic form of *W. bancrofti* was recorded in Andaman and Nicobar Islands16-18. Bancroftian filariasis, in India is transmitted mainly by *Culex quinquefasciatus* which is a night biting mosquito and the mf periodicity is nocturnal except for those reported from Andaman and Nicobar Islands16-19. There is no report on microfilarial periodicity of *W. bancrofti* from Assam; therefore, the present study was undertaken in Dibrugarh district of Assam to observe the pattern of microfilarial periodicity.
MATERIAL & METHODS

Study location and collection of peripheral blood samples

The study was conducted in tea garden worker population of Dibrugarh district, Assam, Northeast India. It lies at a latitude 27°32'26.51''N and longitude 95°15'21.68'' E with an altitude of 397 ft. The number of participants considered in the study was selected on the basis of available literature on earlier studies of microfilarial periodicity and in consultation with biostatistician12, 20-22. In total, 10 mf (W. bancrofti) carriers aged between 18 and 40 yr harbouring moderate to high density of mf in their peripheral blood were recruited after informed written consent. All the 10 participants were hospitalized in the hospital of the tea estate for a period of 30 h. A total of 50 μl of finger prick blood sample was taken from each individual on glass slide at two-hourly intervals (12 collections) using a micropipette. Sample collection started at 1830 hrs and the last collection was done at 1630 hrs on the following day (Table 1). Slides were processed and stained with Giemsa following standard protocol23. Slides were then examined under the microscope for quantification of mf density.

Analysis of data

The method of Sasa and Tanaka24-25 based on goodness of fit to the harmonic wave equation was used for analysis of periodicity data. Assuming that the mf periodicity follows a ‘harmonic wave type’ the relationship between mf density (y) and hour of day (h) was expressed as:

\[ y = m + a \cos 15(h-k)° \]  \( \ldots (1) \)

Where, m, a and k are the mean, amplitude and peak hour density. As 24 h correspond to 360°, the hours 0 to 24 are multiplied by 15 to correspond to angle 0° to 360°.

The harmonic equation of Sasa and Tanaka (1) was simplified by Aikat and Das26 as follows (2) and was used in the present study for estimation of the parameters m, a and k.

\[ y = m + b \cos 15 h + c \sin 15 h \]  \( \ldots (2) \)

Solving equation (2) for b and c we have,

\[ b = a \cos 15 k \]  \( \ldots (3) \)

\[ c = a \sin 15 k \]  \( \ldots (4) \)

Hence, \( a^2 + b^2 = c^2 \) or a (amplitude) = \( \sqrt{b^2 + c^2} \) \( \ldots (5) \)

\[ \tan 15 k = c/b \]  \( \ldots (6) \)

Thus, problem of estimation of m, a and k of (1) is reduced to that of measuring the parameters of (2) and then application of relationships (5) and (6).

Now, the least square estimates of \( m, b \) and \( c \) of equation (2) are as follows:

\[ m(\text{mean}) = \frac{1}{n} \sum y \]  \( \ldots (7) \)

\[ b = \frac{2}{n} \sum y \cos 15 h \]  \( \ldots (8) \)

and

\[ c = \frac{2}{n} \sum y \sin 15 h \]  \( \ldots (9) \)

Table 1. Observed individual microfilarial density, and the observed and theoretical mean mf ratios, at two-hourly intervals for the 10 study individuals

| Individual No. | Age/Sex | Mf density (mf/50 μl) at the hours of examination (hrs) | 1230 | 1430 | 1630 | 1830 | 2030 | 2230 | 0030 | 0230 | 0430 | 0630 | 0830 | 1030 |
|----------------|---------|---------------------------------------------------------|------|------|------|------|------|------|------|------|------|------|------|
| 1              | 20 M    | 0            0            0            32            180            317            439            188            211            33            0            0            0            0 |
| 2              | 40 M    | 1            2            6            225            354            395            565            401            304            78            4            2            0            0 |
| 3              | 18 M    | 0            0            0            33            144            114            136            152            54            83            0            0            0            0 |
| 4              | 18 M    | 0            2            0            85            138            90            156            141            87            91            1            0            0            0 |
| 5              | 20 M    | 1            0            1            10            103            85            106            83            57            43            40            0            0 |
| 6              | 35 M    | 1            2            16           95            82            186            72            76            96            8            3            1            0            0 |
| 7              | 22 M    | 0            1            0            4            25            19            23            19            13            11           0           0           0 |
| 8              | 28 M    | 0            2            4            117           163           186           197           143           104           3           0           0           0 |
| 9              | 25 M    | 0            1            7            58            177           222           276           164           141           16           6           4           0           0 |
| 10             | 24 M    | 0            0            0            5            28            40            49            27            32           23           0           0           0           0 |
| Observed mean mf ratio (Y) | 0 | 1.4 | 4.2 | 91.8 | 193.3 | 229.4 | 280.9 | 190.5 | 151.5 | 54.2 | 1.4 | 1.4 |
| (% of Max)     | (0)     | (0.5)       | (1.5)       | (32.7)       | (68.8)       | (81.7)       | (100)       | (67.8)       | (53.9)       | (19.3)       | (0.5)       | (0.5)       |
| Theoretical mf ratio (Y') | –35.2 | –9.2 | 46.2 | 116 | 181.5 | 225.1 | 235.2 | 209.1 | 153.8 | 84 | 18.5 | –25.1 |
| (% of Max)     | (–15)   | (–3.9)   | (19.6)    | (49.3)       | (77.2)       | (95.7)       | (100)       | (88.9)       | (65.4)       | (35.7)       | (7.9)       | (–10.7)     |
Data were analysed keeping into account of mf ratio and not the actual mf count which eliminated the effect of individual differences in mf intensity as described by Sasa and Tanaka. The mean mf intensity for the 12 blood collections was calculated. The observed mf ratio \( Y \) was calculated for each hour as the percentage of the individual mean mf density for each examination hour divided by the mean mf count. Using the method of Aikat and Das, the values of both \( a \) (amplitude) and \( m \) (mean) were estimated directly. The periodicity index was calculated as relative amplitude or coefficient of variation of observed mf count by, 
\[
\left( \frac{a}{m} \times 100 \right)
\]...

(Ethical considerations)
The study protocol was approved by the Institutional Ethics Committee (IEC) of Regional Medical Research Centre, Dibrugarh, Assam. Investigators explained the objectives of the study to each participant and written informed consent was obtained from each of them. The females were reluctant to participate, hence not included in this study.

RESULTS
Parasitological observation
Results of the two-hourly blood examination of 10 microfilaraemic individuals are shown in Table 1. The observed individual peak hours ranged from 2230 to 0230 hrs. The mean mf ratios for the 10 individuals at each examination time and the mean mf ratios expressed as percentages of the maximum value were calculated (Table 1). The relative periodicity (observed mf periodicity graph) curve (Fig. 1) was drawn by plotting the ratios obtained as the percentage of the individual mean mf density for each examination hour divided by the mean mf count (Y-values shown in Table 1). A clear nocturnal pattern of periodicity was observed, with a peak time around 0003 hrs.

Statistical analysis
The statistical analysis of the periodicity data according to the method of Aikat and Das is presented in Table 2. The analysis indicates a periodicity index of 136.2. In Table 2, 0.5 h corresponds to examination hour 0030, so 0.1 h will be equal to 0006 hrs. Therefore, the \( k \) value of 0.05 h will be equal to examination hour 0003. According to Sasa and Tanaka, \( W. bancrofti \) form involved are of the nocturnal periodicity. From the Y-values of Table 2, the theoretical relation between mf ratios (Y) and hour of the day (h) for the 12 examination times were calculated as per the theoretical mf periodicity equation
\[
Y' = 100 + 103.9 \cos 15h + 54.9 \sin 15h
\]

The theoretical mf ratios (Y’ values) for the 12 examination hours, calculated by this equation, are presented in Table 1. Theoretical mf ratios were subsequently expressed as percentages and also as percentages of the maximum value as shown in Table 1. The theoretical mf ratios (Y’ values) obtained as percentage of the individual theoretical mf ratios for each examination time were used

![Fig. 1: The observed (Y) and Y', the theoretical (Y') W. bancrofti periodicity curves. The curves are based on the observed and theoretical mean mf ratios, respectively, expressed as percentage.](image-url)
to draw the theoretical mf periodicity curve shown in Fig. 1. It is obvious from the figure that the observed and theoretical mf periodicity curves follow each other closely, especially between 2030 and 0430 hrs, and the above equation, thus accurately expresses the relation between mf ratio and time of the day for the study area.

DISCUSSION

World Health Organization has set up a goal for elimination of LF by 2020. India has joined hands with WHO and put the year 2015 as the target year for elimination of LF from India. The main stay of the LF elimination programme is mass drug administration (MDA) which requires monitoring of mf status before each round of MDA through night blood surveys. Ideally, survey done during peak hours of mf density in the peripheral blood of mf carriers provide accurate results of mf status for assessing the effectiveness of MDA programme in a given location. Thus, the information about peak abundance of mf in the human peripheral blood is pertinent and hence, periodicity of mf is to be ascertained. Studies conducted on mf periodicity elsewhere has shown that there are many factors which influence circadian cycle of mf, but vector biting rhythm play an important role in periodicity pattern of mf10-12. In India, three genetically determined physiological races exist for W. bancrofti which are the nocturnally periodic, nocturnally sub-periodic and diurnally sub-periodic forms28.

The state of Assam is located in the Northeastern part of India and is endemic for bancroftian filariasis. Northeastern region of India has unique geographical location and shares international boundaries with Nepal, China, Bhutan, Myanmar and Bangladesh. The evolutionary association of W. bancrofti circulating in Assam is unknown; however, there are reports of two distinct strains of W. bancrofti exhibiting two different microfilarial periodicity from another South Asian country, Thailand29. Further, one strain of W. bancrofti in Thailand is nocturnally sub-periodic while Myanmar strain is nocturnally periodic30. These evidences strengthen the view that presence of different strains of W. bancrofti in a close geographical area is possible.

In our study the peak hour of mf density in the peripheral blood (0003 hrs) was found somewhat coinciding with the peak biting hour of Cx. quinquefasciatus (from 2200 to 2300 hrs) reported in a study in India31. In India, W. bancrofti exhibit nocturnal periodicity and vector involved in transmission is Cx. quinquefasciatus; however, in Andaman and Nicobar Islands of India sub-periodic form of W. bancrofti has been reported and vector involved is Ochlerotatus niveus (day biter)19. Simonsen et al22 while studying periodicity of W. bancrofti in Tanzania reported nocturnal periodicity and discussed importance of sampling time while dealing with epidemiological studies. In the present study, we also recorded presence of mf in the peripheral blood during day time, but at a very low level (0–16) which attained a peak density during night around 0003 hrs. This change in the density of mf was independent of the individual status of mf count of the participants. The similar findings were also observed by Fontes et al22 while studying periodicity of W. bancrofti in western Brazil. It may be a limitation of our study that we did not recruit female participants as they were reluctant and could not consent for this study.

Our finding endorses the nocturnal form of periodicity of W. bancrofti prevailing in Assam and supports the view that most of the Indian forms of W. bancrofti is nocturnally periodic in nature. However, the peak mf count in Assam is about one hour ahead to that in other parts of the country which could be due to the fact that sunset time in Assam is one hour earlier than in western/southern/northern parts of the country. Our findings add a new data on mf periodicity from this region of the country. The outcome of this study is relevant in the context of ongoing MDA programme for elimination of LF from Assam where night blood survey is an important component of LF elimination.

Conflict of Interest

Authors have no conflict of interest with regard to this communication.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the contribution of Dr J. Islam, Medical Officer of the Tea Garden Hospital and the Manager of the Tea Estate for their help and support in carrying out the present study. Technical assistance rendered by Shri NK Baruah and Shri Pranab Saikia are gratefully acknowledged.

REFERENCES


Correspondence to: Dr A.M. Khan, Regional Medical Research Centre (ICMR), Northeastern Region, Dibrugarh–786 001 (Assam), India. E-mail: abdulmaboodkhan@gmail.com

Received: 12 February 2015 Accepted in revised form: 15 April 2015