Prevalence of lymphatic filariasis in a tea garden worker population of Dibrugarh (Assam), India after six rounds of mass drug administration


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

ABSTRACT

Background & objectives: Lymphatic filariasis (LF) is endemic in the state of Assam and mass drug administration (MDA) programme for LF elimination is being implemented in the state since 2004. A study on prevalence of microfilaria (mf), disease endemicity and vector infection was carried out in a tea garden population of Dibrugarh, Assam (India) to assess the effect of ongoing MDA programme on elimination of LF.

Methods: Finger prick thick blood smears (20 mm²) were made from individuals aged ≥2 yr old during night blood survey in between 2000–0000 hrs during the period of November 2012 to February 2013. Blood smears were dehaemoglobinised, stained with Giemsa and examined under microscope for presence of mf. Indoor resting mosquitoes were collected during 0600–1000 hrs and female Culex quinquefasciatus were dissected and examined under microscope for larval forms of the parasite.

Results: A total of 634 blood smears were collected and screened for mf and 47 (7.41%) individuals were found microfilaraemic, with predominance of males (74.5%). Highest mf rate (20.0%) was seen in the males of 30–39 yr age group while in females, age group of 10–19 yr recorded maximum mf rate (5.48%). Entomological collection and dissection of Cx. quinquefasciatus revealed presence of larval stages of the parasite and infection and infectivity rates recorded were 13.20 and 3.70%, respectively. Chronic clinical manifestations in the form of elephantiasis and hydrocele were recorded in 33 (5.73%) subjects of the 575 examined.

Interpretation & conclusion: Mass drug administration data showed six rounds of MDA with drug distribution coverage in between 63.42 and 95.93% in the study population. Out of 634 individuals examined 47 were found microfilaraemic giving an overall infection rate of 7.41%. Mosquito vector infection and infectivity rates were 13.20 and 3.70%, respectively. Presence of high mf rate, vector infectivity rate and clinical cases in the study population after six rounds of MDA warrants concerted efforts to be made for effective implementation and monitoring of MDA for success of LF elimination programme.

Key words Assam; Culex quinquefasciatus; lymphatic filariasis; mass drug administration; microfilaria; Wuchereria bancrofti

INTRODUCTION

Lymphatic Filariasis (LF) is an important parasitic disease affecting mainly socioeconomically poor population of 73 endemic countries as per recent progress report of WHO (2015)¹. Out of these endemic countries, 18 countries are in the advance phase of LF elimination while, 55 countries are still continuing MDA. Contribution of India to disease burden in Southeast Asian countries is about 73%¹. In India, LF is mainly caused by Wuchereria bancrofti and Brugia malayi form of the filarial parasite. Out of 250 districts covered under MDA, 12 districts have stopped MDA and another 95 districts are preparing for transmission assessment survey (TAS) in the coming days².

Tea industry is an important cash crop industry of Assam. In earlier studies, high prevalence of LF was recorded from tea worker population of Assam³/sys. MDA programme is being carried out in various endemic areas of Assam⁸. Therefore, considering high prevalence of LF among tea garden worker population and ongoing MDA programme for elimination of LF, an epidemiological survey dealing with prevalence of microfilaraemia, clinical observation, and vector infection load was carried out in a tea garden population of Dibrugarh. Tea worker population mainly constitutes descendants of migrants brought from Bihar, Jharkhand, Odisha, West Bengal and Uttar Pradesh during pre-independence period by Britishers to employ in tea industry. This population is confined to the tea industry and resides in densely populated workers’ colonies, generally called as labour-lines. They maintain their socio-cultural practices. Further, houses in the labour lines are made up of thatch or tin roof with mainly brick walls. Sizeable populations of tea garden workers are illiterate with few having matriculation and above high school level of education. Females
are less educated than males. Per family monthly income of the tea workers is approximately below ₹5000.00.

Workers engaged in plucking tea leaves works from 0500 to 1530 hrs, while those working in the factory work during late night hours. Consumption of locally made liquor is common practice among the tea garden population.

**MATERIAL & METHODS**

**Study area and period**

The study was conducted in a tea garden (Udalguri tea estate) located in the District of Dibrugarh, Assam. It lies at a latitude 27°32' 26.5''N and longitude 95°15' 21.6''E having an altitude of 397 ft. This study was undertaken during November 2012 to February 2013. The garden comprises of 242 households with a population of 1460 according to 2012 census done by the tea garden management.

People are of low socioeconomic group and most of them work in the tea garden. There are eight labour-lines in the garden and each labour-line comprises of 20-30 households. Generally houses are made up of brick walls with tin roofing provided by the tea company. However, often extension of rooms is done with clay wall, bamboo and thatch/tin roofing. Tea garden hospital lies near the lines and cater health care facilities to tea garden workers. Houses in labour-lines are densely situated and sanitary facilities are poor. Water supply is provided by hand pumps (tube-wells) installed in the labour-lines. Due to poor drainage system, stagnant water near the tube-wells supports profuse mosquito breeding in the labour-lines.

**Ethical consideration**

The study protocol was approved by the Institutional Ethics Committee (IEC) of Regional Medical Research Centre, Dibrugarh. Investigators explained the objectives of the study to the participants and obtained informed verbal consent/assent from all the participating individuals.

**Night blood survey**

Before night blood survey IEC activities were jointly done by the research team and tea garden hospital management. Night blood survey was done between 2000 and 0000 hrs by collecting finger prick blood samples from participating individuals (≥2 yr of age). Thick blood smears using 3–4 drops of blood were made for each sample. Samples were dried and brought to the laboratory for parasitological examination. Information about age, sex, history of previous treatment with DEC/albendazole was obtained from each individual. A total of 634 finger prick blood samples were collected during the survey.

**Parasitological examination**

Dried thick blood smears were dehaemoglobinised, fixed in methanol and stained with 10% Giemsa solution. Stained slides were examined under microscope (10x) for the presence of microfilaria (mf). Microfilaria number was counted in each positive slide and recorded.

**Clinical examination**

Clinical examination of selected individuals (≥18 yr of age; as clinical manifestation in LF is normally evident after adulthood) was done and chronic cases of hydrocele and elephantiasis were recorded. Moreover, subjects found mf positive in the night blood survey were also clinically examined irrespective to their age. Disease rate in the garden was calculated.

**Entomological investigation**

Entomological collections (indoor resting) were done in randomly selected houses (10 numbers) in the labour lines during morning (0600 to 1000 hrs) using suction tube and flash light in the month of January 2013. Caught mosquitoes were brought to the laboratory and examined morphologically under binocular microscope. Female *Cx. quinquefasciatus* were identified using standard mosquito identification keys; dissected using standard procedure and separated into head, thorax and abdomen on a glass slide; and tissues were cut and separated apart to locate the presence of larval forms (*L*₁/*L*₂/*L*₃) of the parasite. Dissected mosquito(s) having any larval form of the parasite were recorded positive. Mosquitoes having *L*₁, *L*₂ and *L*₃ (either any or all) forms of the larvae were treated as infected mosquitoes while mosquitoes having *L*₃ larvae with or without other larval forms of the parasite were regarded as infective mosquito. Number of larvae present in the mosquitoes were counted and recorded. Per man hour density (MHD) of collection of female *Cx. quinquefasciatus* at study site was calculated by using the formula:

\[
\text{MHD} = \frac{(n \times 60)}{(t \times P)}
\]

Where, \(n\) = Total number of mosquitoes collected; \(t\) = Time spent in min; \(P\) = Number of persons involved.

**Statistical analysis**

Data generated in the study were analyzed using Excel spread sheet and SPSS 17.0 software. The mf rate was calculated as the ratio in percentage of total number of persons found positive to the total number of persons examined.
RESULTS

**MDA programme in the study tea garden**

The tea garden under study comes under the District of Dibrugarh and MDA for elimination of LF was being observed in the district since 2004. As the tea garden does not fall under sentinel population, data on mf rate, vector infection rate and drug compliance (drug intake) was not available.

**Prevalence study**

Of the total 1460 tea garden population, 634 (43.42%) individuals were surveyed for mf prevalence (320 males and 314 females). Forty seven (47) individuals were found microfilaraemic giving an overall infection rate of 7.41%. The age group-wise prevalence of microfilaraemia in the population has been shown in Table 1. The mf rate was highest in males in the age group of 30–39 yr, while in females highest mf rate was recorded in the age group of 10–19 yr (Table 2). The lowest infection rate was recorded in the age group of 50 yr and above in males and 30–39 yr in females. Microfilaria rate (10.94%) was significantly higher in case of males compared to females (3.82%). The highest mean mf density was recorded in the age group of 40–49 yr in males and 30–39 yr in case of females (Table 2). The lowest mean mf density was recorded in the age group of 50 and above in males and 40–49 yr in females. The frequency distribution of mf count indicates that majority of infected individuals have low mf count in their blood. More than 19% of infected individuals exhibited one mf/20 µl of blood. However, in 2007, after three rounds of MDA, mf rate for the whole Dibrugarh district recorded was 3.89%. Data collected from the tea garden management on MDA are summarized in Table 3. The drug distribution coverage ranged from 63.3 to 95.93% across the MDA programme, however, data on actual compliance for drug intake is not known. The proportion of subjects with high microfilariae count (>10 mf/20 µl) was around 2.6% of the total infected subjects (Fig. 1).

<table>
<thead>
<tr>
<th>Table 1. Age-wise mf rate and density in the study population</th>
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<td><strong>Age group</strong></td>
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<th>Table 2. Age-wise mf rate among male and female population</th>
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<td><strong>Age group (yr)</strong></td>
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<td><strong>Population examined</strong></td>
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<td><strong>% Population covered</strong></td>
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<td><strong>No. positive</strong></td>
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<td><strong>Microfilaria rate</strong></td>
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<td><strong>Mean mf density</strong></td>
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M–Male; F–Female.
Parasitological examination

Stained slides were examined under microscope (10×) for the presence of mf and morphologically identified as *W. bancrofti* with its identifying characteristics as shown in Fig. 2.

Clinical study

A total of 575 individuals were clinically examined and of these 33 were found to be having either elephantiasis or hydrocele thus showing the disease endemi city rate of 5.74%. Of the 33 clinical cases, only 3 (9.09%) were having elephantiasis. All the three elephantiasis cases were in the age group of 36–45 yr. Of the three elephantiasis cases, one was female and the other two were males. Most of the cases of hydrocele were in the age group of 16–25 yr (36.66%) followed by age group 26–30 yr (30%). Three hydrocele cases were observed as early as in 16 yr of age.

Entomological study

A total of 84 mosquitoes were collected from 10 randomly selected houses of which 53 (63.10%) were *Cx. quinquefasciatus*. The dissected mosquitoes were found to harbour *W. bancrofti* larval forms, i.e. L1, L2 and L3 stages (Fig. 3). Vector infection and infectivity rate was 13.20 and 3.70%, respectively (Table 4). The per man

![Fig. 2](image1.png) (a) Giemsa stained microfilaria of *W. bancrofti* with details of internal structures (40×). CS–Cephalic space; NC–Nuclear column; NR–Nerve ring; EP–Excretory pore; EC–Excretory cell; IK–Innen korper; R1–R4–Rectal cells; AP–Anal pore; TN–Tail nuclei; TS–Tail space; and (b) Haematoxylin stained microfilaria showing sheath distinctly (40×).

![Fig. 3](image2.png) Larval stages of *W. bancrofti* in the vector mosquito (*Culex quinquefasciatus*); (a) L1 stage; (b) L2 stage; and (c) L3 stage. Magnification: 40×.

<table>
<thead>
<tr>
<th>Site of collection</th>
<th>Mosquito collection</th>
<th>Female <em>Cx. quinquefasciatus</em> collected/dissected</th>
<th>No. of mosquito found positive for larval stages (L1/L2/L3)</th>
<th>Larval stage found</th>
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hour density (MHD) of collected female *Culex quinquefasciatus* was 4.42.

**DISCUSSION**

Single annual dose of MDA for elimination of LF was launched in 2000 by the WHO under an ambitious Global Programme to Eliminate Lymphatic Filariasis (GPELF) across the globe. The target set for total elimination of LF is 2020. Two hundred and fifty (250) districts of India are endemic for LF and 600 million people are at risk of infection. India has adopted a Government Health Policy in 2002 and set 2015 as a target to eliminate LF from its territory. For all eligible endemic districts in India, administration of a single annual dose of DEC was started in 2004 which was later (from 2007 onwards) complemented with albendazole (DEC+Albendazole) as combination therapy. However, public mobilization to improve the compliance is still the main bottleneck in the success of ELF programme.

The national average mf rate in 2004 was 1.2% which declined further to 0.35% in 2011. Out of 250 endemic districts of India which were under MDA in 2007, 177 districts were reported with <1% mf rate while 73 districts recorded mf rate of >1% in 2008. The Assam state had reported over 9.4 million people at the risk of infection, 0.36 million mf carriers and 90 thousand clinical cases during 1991. MDA in Assam was started in 2004 and presently seven of the 28 districts are covered under MDA programme. Dibrugarh district is under MDA since 2004 and assessment of effectiveness of MDA programme done during 2007–10 has shown declining trend of mf rate.

In the Dibrugarh district more than six rounds of MDA have been completed. Present survey shows an overall mf rate of 7.41% which is quite high after six rounds of MDA and younger age group found positive for mf indicates continuity of the transmission of infection. Highest mf rate of 11.22% was noted in the age group of 30–39 yr. MF rate in male was quite high compared to female. Chances of higher exposure of male subjects to infective mosquito bite could be due to clothing practices where more body parts are exposed to mosquito bite compared to females. Further, in male, practice of consumption of locally made liquor is quite high compared to females and during early night hours which also coincide with biting cycle of *C. quinquefasciatus*, males under the influence of alcohol become least bothered to mosquito bite and hence this might have resulted into higher exposure to mosquito bite culminating into high mf rate.

Entomological findings also suggest continuity of transmission as high infection and infectivity rate were found during the study (Table 4). The study was carried out in the months of winter (November 2012 to February 2013) when vector density of *C. quinquefasciatus* remains at low level. We have not done survey during the period of monsoon so, vector density and transmission level during monsoon is not known. Probability of higher transmission level during monsoon than winter months may not be ruled out.

During the survey, stagnation of domestic waste water in the form of small water pools was recorded around the deep tube-well installed in the worker residential lines. These water bodies provide favourable conditions for profuse mosquito breeding.

Data on frequency distribution of mf in mf carriers (Fig. 1) showed that most of the mf positive individuals had low mf count and this is normally reported in the population where inadequate dose of antifilarial drugs is received under MDA programme.

Clinical manifestation of LF observed as 5.74% and the presence of several hydrocele cases in younger population (16–25 yr) further supports the view of continuity of transmission of the infection and poor drug compliance in the population. Initially clinical examination was conducted in ≥18 yr of individuals, however, all mf carriers irrespective of the age were also clinically examined, though normally onset of clinical manifestation starts after the age of adolescent. However, it would have been better if children of the lower age were also included in the clinical examination as younger age groups were found with clinical disease.

Data available on coverage of MDA on tea garden population from 2007 to 2013 were analysed and tabulated (Table 3). During the year 2007–11 high coverage (drug distribution) was recorded (>80%) and in later years (2012–13) it was between 60 and 70%. Data on individual coverage of MDA are not available with us; hence, age wise coverage of MDA can not be ascertained. However, we analysed the labour-line-wise coverage of data available with us which showed no significant difference in coverage of labour-lines in the studied tea garden. Actual compliance (intake of drug) for our study is not known and often compliance reported elsewhere is much lower than the coverage during MDA.

Although, the drug coverage/distribution was fairly good in the study population, actual compliance was probably much less as tea garden workers routinely use to go for work in the early morning for tea leaf plucking or to the factory and miss the drug distributor. As a result drug distributors generally hand over the medicine to anyone present in the house. Moreover, a drug distributor has to cover/distribute drug to a minimum of 250 individuals per
day, he/she may not be serious in monitoring the swallowing of the drug. Though, top up rounds were also observed during MDA that still may only increase the coverage and not the substantial compliance to the drug intake. These could be factors for non-compliance of the drug in the tea worker population. Similar observation was seen in Ghana where it has been categorically emphasized that one or two visits of drug distributor are not sufficient to improve the level of compliance\cite{17}. Although no serious side effects of MDA were reported in the tea worker population, but as per the feedback received from the tea garden health officials, nausea and abdominal cramp were commonly noticed side-effects. The drowsiness effect of DEC which is more generalized in nature might have kept tea workers away from swallowing the drug with the fear that it might hamper their next day work and loss of daily wage. Kumar \textit{et al}\cite{18} has reported that actual compliance of drugs, i.e. 65% or more for the six years is sufficient to eliminate LF, however, it has been seen that gap between actual compliance and drug coverage (distribution) is very wide\cite{19}.

In another study, Krentel \textit{et al}\cite{20} reported that training and motivation of drug distributors is critically important as they directly interact with the target population and can greatly influence the decision of families and individuals regarding compliance to MDA; where compliance is often associated with individual’s perception of the potential risk and benefits ratio versus risk of adverse events. Further, persistency of microfilaremia after several rounds of MDA has also been reported from other LF endemic countries where >1% vector infection rate was sustained after several rounds of MDA\cite{18}. In the Tonga region of Tanzania even eight rounds of MDA were not sufficient to bring down the infection below 1%, as a result it was also suggested to adjunct the MDA with vector control measures like bed net usage and with emphasis on more IEC activities particularly in the male population\cite{20}. In India also high mf rate has been recorded after nine rounds of MDA\cite{21}. In earlier studies we have shown that LF is mainly concentrated in the tea garden worker population\cite{22} and after six rounds of MDA in the tea worker population, mf rate and vector infectivity rate remained quite high. Therefore, the tea garden worker population needs more attention to meet desired level of drug compliance.

IEC activities at tea garden management level may make a difference in drug compliance level among tea garden workers. Therefore, IEC activities done during this study have made the tea garden managements more aware about the LF elimination programme. Further, it was stressed that MDA coverage and compliance should be scaled up in pediatric group by state programme manag-
ers. Tea Estate management are now actively participating in the MDA programme. Small \textit{et al}\cite{23} were of the views that \textit{W. bancrofti} strain(s) circulating in an endemic area also pose hindrance to the success of MDA programme. Some of the strains of \textit{W. bancrofti} may not be adequately susceptible to DEC and albendazole as several haplotypes of \textit{W. bancrofti} have been reported from LF endemic areas. Researchers are of the view that effect of MDA on LF control/re-appearance of mf after reaching the threshold level needs to be seen from the point of view of genetic complexity of \textit{W. bancrofti}\cite{23-24}.

Finally, though in Assam number of MDA districts are small and programme is manageable for the required period (6 yr or more) elevation of drug compliance (intake) level is the foremost factor to achieve complete success in LF elimination. Authors are of the view that better compliance to MDA can only be achieved when target community is well-informed and drug distributors give emphasis to drug intake. IEC activities coupled with increased number of mop-up rounds of MDA are also important components of the ELF success\cite{25}. Timings of the drug distribution are also responsible for poor drug compliance in tea garden setup. Further, tea industries in Assam have hospital and health worker facilities and hence, they should be included in the MDA programme. For mop-up rounds drugs can be given to the hospitals of the tea gardens which can be made available to uncovered population over a period of time. This will not involve extra expenses on the part of the primary health centres (PHCs) which often experience fund crunch for additional mop-up rounds of MDA.

\textbf{CONCLUSION}

LF elimination in the State of Assam is achievable but poor drug compliance is the main bottleneck to the elimination programme. Community participation in adjunct to training of drug distributors for meeting the target of drug compliance are the essential components in the success of ELF.

\textbf{Conflict of interest}

The authors declare that they have no conflict of interest.

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Correspondence to: Dr A.M. Khan, Regional Medical Research Centre (ICMR), NE Region, Post Box No. 105, Dibrugarh–786 001, Assam, India.
E-mail: abdulmaboodkhan@gmail.com

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