



Epidemiological screening and xenomonitoring for human lymphatic filariasis infection in select districts in the states of Maharashtra and Karnataka, India

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Abstract

Lymphatic filariasis (LF) is a mosquito-transmitted tropical neglected parasitic infection that currently affects over 120 million people around the world and another 856 million people are at risk of acquiring the infection. Mass Drug Administration (MDA) spearheaded by the World Health Organization is the only current strategy to control this infection in endemic areas. In this study, we performed an epidemiological survey in select regions in the southern parts of India to determine the current status of LF infection in subjects. Night blood samples were collected from 916 subjects after proper consent and were screened for the presence of circulating microfilariae of *Wuchereria bancrofti* in their peripheral blood. Our results showed the presence of 51 (5.56%) cases of human LF infection in the surveyed areas including new cases for LF, which were not recorded previously. Given the presence of new cases of LF infections, we trapped mosquitoes from these regions and screened for the presence of *W. bancrofti* L3 specific *Ssp1* DNA repeat sequences by PCR. Our results confirmed the presence of LF infection in the mosquitoes collected from six out of nine districts that we surveyed. These findings confirm active transmission of LF infection in all of the areas that we surveyed, despite several years of MDA treatment. The findings in this study suggest potential reemergence of LF infection in most of the areas we surveyed and warrants for a more stringent strategy for controlling LF in these endemic areas.

Keywords Lymphatic filariasis · Mosquitoes · PCR · Night blood screening · Reemergence of infection · India

Introduction

Lymphatic filariasis (LF) is a major public health problem in tropical areas, caused by one of three filarial parasites;

Wuchereria bancrofti, *Brugia malayi*, and *Brugia timori* (WHO 2018). The disease transmission occurs through a bite from infected mosquitoes that carry infective larvae of LF. According to World Health Organization, over 120 million people are infected with LF; of this, about 40 million suffer from severe physical disability, which include 25 million hydrocele cases and 15 million lymph edema cases of the leg (WHO 2018). Another 856 million people are at risk of acquiring the LF infection (WHO 2018). A major strategy to control LF infection in the endemic regions is currently the mass drug administration (MDA) approach, where a combination of two drugs diethylcarbamazine and albendazole or ivermectin is given annually to all the individuals living in an endemic area (WHO 2010). Published data to date suggest that MDA is the most effective approach in limiting or interrupting the transmission of LF in a community (Brady 2014; Ramaiah and Ottesen 2014). Despite its tremendous success, subject non-compliance appears to be the major drawback in the MDA approach to control the infection (Babu and Kar 2004; El-Setouhy et al. 2007; Krentel et al.

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2013; Kumar et al. 2009; Nujum et al. 2012). If subjects fail to take the medication, the risk for infection increases due to the huge mosquito population in these areas and presence of infected individuals in the community. A second drawback is that the drugs used in the MDA have little or no effect on the adult worms living inside the lymphatics. Thus, once the effect of drug wanes, the adult parasite starts producing the microfilariae that appear in the peripheral blood further increasing the risk of reinfection. These drawbacks are believed to play a significant role in the higher risk of reemergence of the LF infection in a community despite the yeomen's efforts to control the infection with MDA (Krentel et al. 2013; Nujum et al. 2012; Silumbwe et al. 2017; Sunish et al. 2014). Similarly, migration of infected population into a community can also play a significant role in the emergence and resurgence of LF (Toothong et al. 2015).

In this study, we surveyed select endemic regions within India to determine the status of LF infections. Indian sub-continent collectively contributes to almost 44.3% of the global LF burden (WHO 2010) with about 610 million people currently residing in the endemic areas and are exposed to the infection (Raju et al. 2010). Subjects in the select regions that we surveyed received MDA for the past 10 years or more (NVBDCP 2014). The reports from the National Vector Borne Disease Control Program (NVBDCP) of India documents that in 1995, over 6% of subjects living in these surveyed areas was positive for microfilaria (Mf). This rate of infection remained almost the same (1–5%) up to 2003 (NVBDCP 2014). Subsequently, when the MDA campaign was started, the population of Mf positive subjects dropped to about 1.13–1.87% in 2004. The data from 2014 showed that the population of Mf positive subjects remained steady at 0.53–1.19% (NVBDCP 2014). The reason for the existence of the residual infected population in these communities despite several rounds of MDA is unknown. Nevertheless, the MDA approach was highly effective in reducing the transmission. Unfortunately, in most of the villages where the LF infection is endemic, there is a huge mosquito population making it difficult to control the infection with therapeutic drugs alone. Thus, any residual infected population may become the “hot spot” for the spread of infection locally that could eventually lead to reemergence of the infection if proper control measures are not instituted. There has been no systematic approach to identify LF “hot spots” in India. Therefore, in this study, we selected several key districts that were highly positive for LF infection before the start of the MDA program and wanted to assess the status of LF infection in these districts after 10 years of MDA. We screened the night blood samples of apparently healthy subjects residing in several areas within each district to determine the presence if any of LF microfilariae (Mf) positive subjects. We also trapped mosquitoes from these regions to determine if any mosquitoes

carry the LF infection. Our results demonstrate active LF disease transmission in all the areas we screened.

Materials and methods

Ethics statement

Use of human subjects in this study was reviewed and approved by the Institutional Review Boards of the Mahatma Gandhi Institute of Medical Sciences, Sevagram, India, University of Illinois Rockford and the Indian Council of Medical Research Regional Center, Belgaum India. Blood samples were collected from each subject after proper informed written and oral consent in local language. We also have the minor assent and parental permission with the written consents in local language for all the children who participated in this study. Inclusion criteria for enrollment of subjects in this study were both male and female subjects under the age of 71 and children above 2 years of age. Exclusion criteria for this study were both male and female subjects above 71 years of age, children under 2 years of age, patients with chronic LF infection showing visible lymphedema in their legs, and children with hydrocele. Chronic patients with lymph edema and hydrocele were identified by the public health worker before enrolling them into the study.

Screening endemic areas in India for current LF transmission

During September through December of 2014, we screened the night blood samples of 916 human subjects for the presence of *W. bancrofti* Mf. About 60% (550) of the subjects were male and about 40% (366) of the subjects were females. About 20% (183) of the samples were from children between the ages of 3 and 21. Based on the public health record, the screened villages were highly endemic (> 6% incidence) for lymphatic filariasis before the start of MDA in 1995 (Indian Council of Medical Research). We selected the districts based on the MDA distribution record from the public health department. The surveyed districts were in Maharashtra and Karnataka states of India (Fig. 1). The survey team visited the areas in the evening and blood samples were collected after 20:00 h. Fresh blood samples were spot checked under a microscope for the presence of actively moving microfilaria (Das et al. 2005). Briefly, 10–20 µl of blood collected by finger prick method was spotted on to a glass slide and examined under the bright field microscope for the presence of live Mf. The public health worker evaluated each enrolled subject for their general health status before blood collection. Subjects who were positive for LF infection by the spot screening were

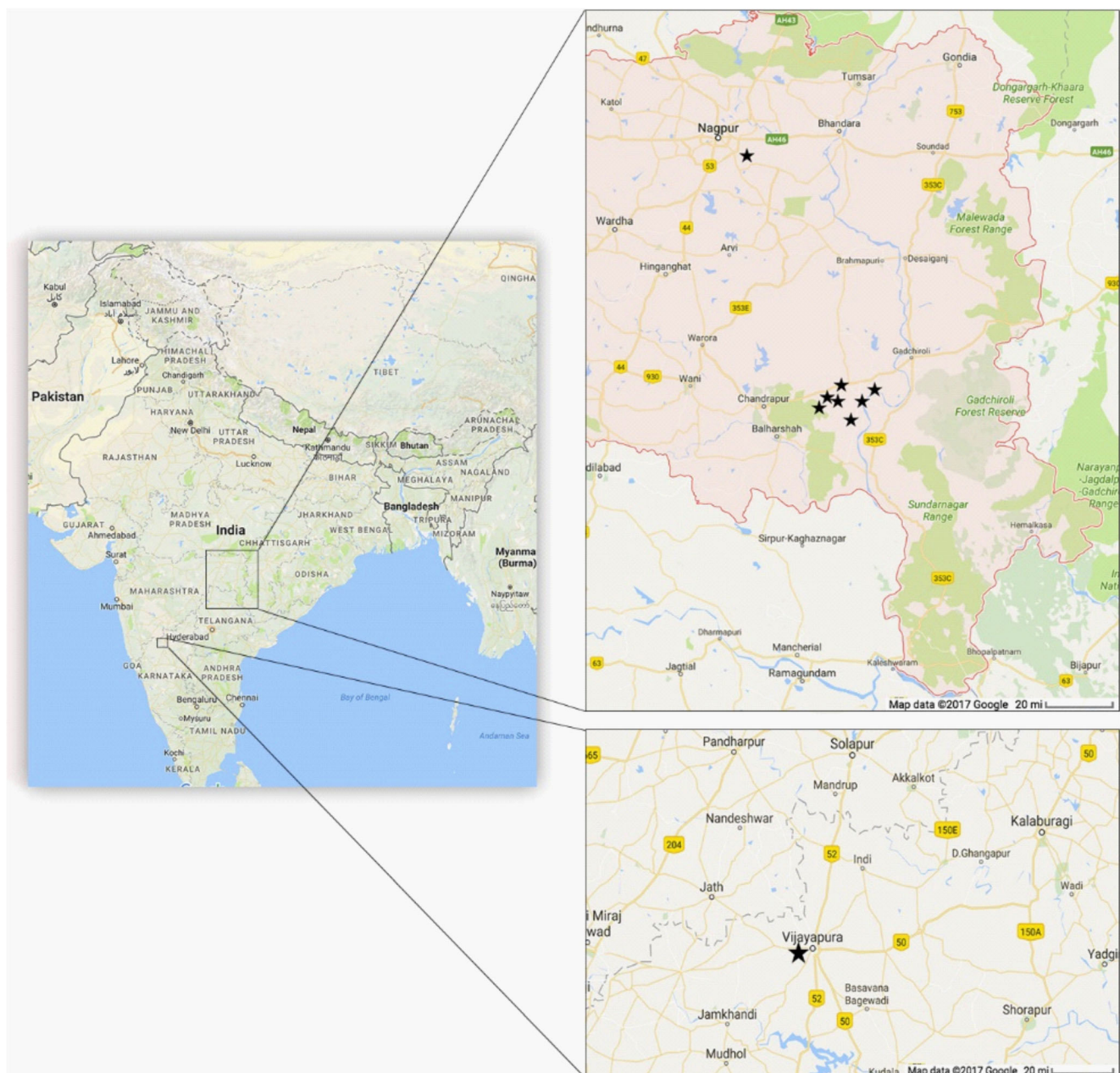


Fig. 1 Geographical presentation of the screened filarial endemic areas in India. The screening of the night blood smear was carried out in the selected areas from India which are known to be endemic for LF and

where MDA has already been implemented. The areas are marked with asterisk sign in the map

treated with diethylcarbamazine (DEC) 6 mg/kg body weight as per the recommendations (NVBDCP 2014).

Screening mosquitoes for the presence of *W. bancrofti* *Ssp1* repeat sequences by PCR analysis

Since we found several new cases of LF infection in all the districts we visited, we wanted to determine if there is any active LF transmission in these areas. We trapped mosquitoes from several locations within each area using CDC miniature light trap (John W Hock Company, Gainesville, FL). The traps

were hung on the porch of several randomly selected houses within the area from 18:00 h to 9:00 h next day. Each trap had over 300–500 mosquitoes. Trapped mosquitoes were frozen down and brought to the laboratory for DNA isolation. After removing the wings, DNA was isolated from groups of 10 mosquitoes each using Genelute blood genomic DNA isolation kit (Sigma Aldrich, MO) and PCR analyses were performed using primers specific for *Ssp1* as described previously (Alhassan et al. 2015; Chambers et al. 2009) for detecting the presence of the infective larvae of *W. bancrofti* in the mosquitoes. The PCR product was then analyzed on ethidium

Table 1 Several lymphatic-filariasis-positive individuals were identified during spot screening of blood samples from subjects living in several regions of India

Region (district)	Blood samples were screened from the subjects for the presence of Mf by night blood smear examination	
	Total individuals screened	Positive for Mf (%)
Junasurla (Chandrapur)	190	11 (5.78)
Navegaon Bhujla (Chandrapur)	84	3 (3.57)
Tolewahi (Chandrapur)	80	7 (8.7)
Chiroli (Chandrapur)	111	5 (4.5)
Usegaon (Chandrapur)	116	11 (9.4)
Kelzar (Chandrapur)	92	6 (6.5)
Kuchadi (Nagpur)	93	3 (3.2)
Mul (Chandrapur)	81	3 (3.70)
Bijapur (Bijapur)	69	2 (2.89)
Total	916	51 (5.56)

bromide stained 1% Agarose gel. Amplified PCR product was sequenced to confirm the *Ssp1* repeat sequences.

Results and discussion

Active transmission of LF occurs in select villages in India where MDA was given for the past 10 years

Our results showed that active LF infections are present in all the regions we surveyed (Table 1). Out of the 916 blood samples screened, 51 samples (~5.56%) were positive for circulating microfilariae. Both male and female subjects including children were positive for the infection. Among the 51 samples, 50% of infected subjects were males, 30% of infected

subjects were females and 20% of infected subjects were children. Age group of infected subjects ranged between 3 and 50 with no clear distribution pattern for infection within various age groups. When we compared our data with the published record of LF infection for the region (NVBDCP 2011; Raju et al. 2010), it was found that there were more positive LF cases than those reported previously. These findings suggest the presence of newer cases of LF infections in these villages. Presence of new infections suggested the possibility of active LF transmission in these areas. To confirm this, we trapped mosquitoes (Fig. 2a) and screened them for the presence of *W. bancrofti* L3 *Ssp1* repeat sequences by PCR. Mosquitoes collected from six out of nine districts were positive for the *Ssp1* gene confirming the presence of active transmission in these areas (Fig. 2b).

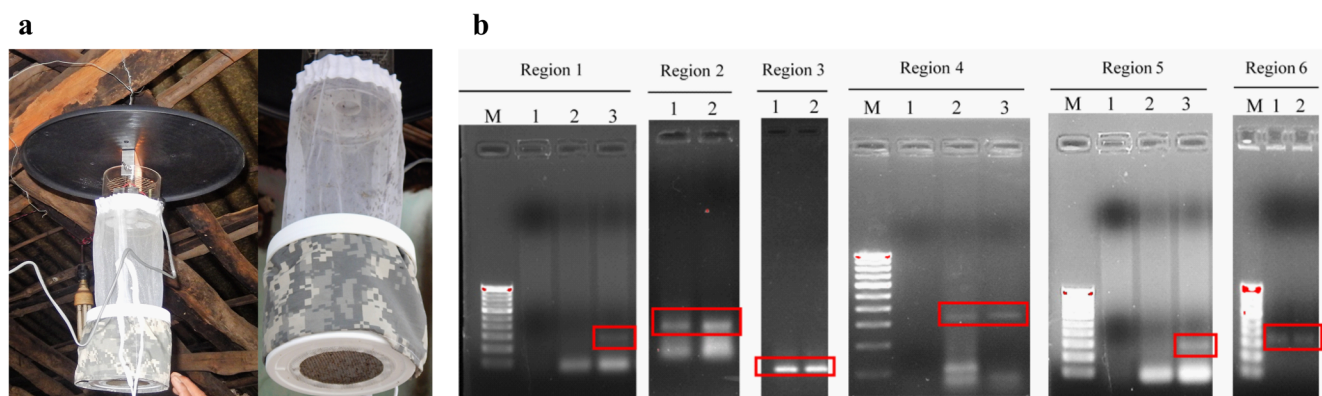


Fig. 2 Mosquito samples from six out of nine regions were positive for *W. bancrofti* specific *Ssp-1* repeat sequences PCR analysis. **a** One of the CDC minitraps removed from an area shows several trapped mosquitoes. **b** DNA was isolated from the mosquitoes and PCR reaction was

performed using *Ssp1* repeat sequence specific primers. The PCR product was analyzed on 1% agarose gel. Lane M; 1 kb molecular weight marker, Lanes 1–3; *Ssp-1* PCR product

Thus, findings from this study showed several “hot spots” of LF infection in India suggesting active transmission of ongoing LF infection. Diethylcarbamazine (DEC) was given to all the “at-risk households” in these districts as part of the MDA campaign by the public health workers (NVBDCP, Indian Council of Medical Research). However, when we visited these households to hang the mosquito traps, we were surprised to find that several households did not even take the medication given to them for several years. Thus, subject non-compliance appears to be one of the major factors in the continued transmission of the infection in these areas. Most alarming was the presence of new cases of LF in these areas that correlated with the presence of infected mosquitoes. When asked, each of the newly identified infected individuals stated that they took the DEC medication given by the public health workers, yet they ended up getting the infection. A possible explanation for this may be that these individuals were exposed to infected mosquitoes after the effect of DEC has waned. DEC is rapidly absorbed following oral administration and peak plasma concentrations are reached within 1 to 2 h (Hawking and World Health Organization 1978). The normal half-life for the elimination of DEC in a healthy individual typically ranges from 10 to 12 h (Awadzi et al. 1986). Thus, if somebody is bitten by an infected mosquito 24 h after taking DEC, their chances of getting LF infection is increased. Thus, as the therapeutic concentration of DEC decreases from the blood, the subjects are at higher risk of acquiring the infection, especially when infected mosquitoes are present. A second possible explanation is that even in infected individuals DEC has very little effect on the adult parasites that are living inside the lymphatic vessels (Babayan et al. 2012; Bennuru and Nutman 2009; Ramaiah et al. 2009). Therefore, Mf produced by these adult parasites starts appearing in the peripheral blood as the effects of the drug wanes. Mature adult female parasites survive for almost 10 years in the host producing Mf, thus necessitating the annual MDA treatment (Ramaiah and Ottesen 2014).

Post-MDA, there have been several reports indicating the resurgence of LF through the Transmission Assessment Surveys (TASs) carried out in India and from other filarial endemic countries (Anil 2012; Bhattacharjee 2016; Patel 2012; Sunish et al. 2014; Zolnikov 2018). Although not reported in the human, the problem of development of drug-resistance in LF can have profound consequences on the success of MDA program (Schwab et al. 2005; Schwab et al. 2006; Wiwanitkit and Wiwanitkit 2011). These findings clearly show that there is a need for a more sustainable and effective approach such as a prophylactic vaccination to prevent LF infection. A multipronged combined approach that includes MDA, an effective prophylactic vaccine and an effective vector control strategy is critical for the success of LF control and elimination.

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