Detection of Vector Mosquito of Filariasis in the Endemic Areas of Bangladesh

Jasmin Nur, Bilkis Sultana, Mohammad Hefzur Rahman, and Mohammad Moniruzzaman

ABSTRACT

Background: Lymphatic Filariasis (LF), a mosquito born tropical parasitic disease, caused by *Wuchereria bancrofti* is not only a health but also a socio-economic problem in Bangladesh. *Culex quinquefasciatus* is the confirmed filarial vector in Bangladesh, so by controlling of this vector population, we can prevent this disease. To control this vector and decrease incidence of filariasis, we need to know studied mosquito's sample in endemic areas.

Aim and objectives: Aim of this study was to see mosquito population in search of vector species in the endemic zones of malaria and filarial. Then we confirmed presence of vector species at molecular level by species specific DNA fingerprinting.

Methods: This cross-sectional entomological study was carried out in Hobiganj (Shatchori tea garden) and Moulvibazar district (Patrokhola and Madonmohonpur tea gardens). Mosquito was collected by paper cup with net, aspirator and torch light and vector mosquitoes were identified with stereoscopic microscope. After identification of the mosquitoes, these were stored in laboratory for analysis of the density of the vector mosquitoes in the endemic areas. Then the vector mosquitoes were identified through molecular method named Polymerase Chain Reaction (PCR).

Result: 1427 female mosquitoes belonging to 28 species under 5 genera were collected from three tea gardens. *Culex quinquefasciatus* which is the confirmed filarial vector in Bangladesh was found high in number (20.74%) out of the total collected mosquitoes. Other mosquito's species were found in various ratios. Then *Culex quinquefasciatus* vector was identified by PCR.

Conclusion: As vector mosquitoes of LF are available in these tea gardens, the study concludes that these areas are highly LF endemic areas of Bangladesh. Through molecular method, the vector of LF can be identified certainly. As we identify the vector mosquito, thus we can prevent this LF disease at very early stage. So, these findings will be very beneficial and cost effective for a developing country like Bangladesh.

Keywords: *Culex quinquefasciatus*, Lymphatic Filariasis, Polymerase Chain Reaction.

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I. INTRODUCTION

A vector-borne disease is that in which the pathogenic microorganism is transmitted from an infected individual to another one by an arthropod or other agents, sometimes with other animals serving as intimidator host. Although invertebrate such as mosquitoes and ticks play the main role for diseases transmission, but some vertebrates may also act as vectors. Filariasis is a mosquito born tropical disease which is very harmful and causes endemic disease. Although various thread-like nematode (worm) parasites and their larvae belonging to the family Filarioidea are responsible for

this disease, *W. bancrofti* is the main causative agent of Filariasis. Different mosquito's species like *Culex*, *Anopheles* and *Mansonia* species are the vectors of this Filariasis. Among these *Culex quinquefasciatus* is the main vector of this disease. Approximately 120 million people in the tropical and subtropical areas of Southeast Asia, South America, Africa, and the islands of the Pacific are affected by various debilitating parasitic diseases [1]. Among them Lymphatic Filariasis (LF) is predominant. About 90% of LF infection occurred worldwide due to *W. bancrofti* and rest 10% for *B. malayi* [2]. LF is occurred when the adult worms distort the lymphatic vessels and cause local inflammation

and swelling. In case of LF, symptoms have shown many years later. Around 10 to 15 years repeated exposure to the parasite of a person LF is established. In case of these diseases 10 to 50 percent of men suffer from genital damage, especially hydrocele (fluid-filled balloon-like enlargement of the sacs around the testes) and elephantiasis of penis and scrotum. These manifestations are mostly irreversible and cause of socio-physical problem to patients and often their families. Chronic lymphatic filariasis causes severe functional impairment and disability [3]-[5] and loss of productivity [6].

Bangladesh is a highly endemic area for LF. As Bangladesh is an overcrowded area and most of the people in this country are poor and live in unhygienic condition, LF is a major public health problem in Bangladesh. It makes them incapable to earn and help their own family. Genital damage especially hydrocele and elephantiasis of scrotum in men and of the breasts in women occur much more frequently. *W. bancrofti* is the only causative agent of LF in both rural and urban areas in Bangladesh and *Culex quinquefasciatus* is the only vector of this parasite [7], [8]. About 70 million people in 32 of the 64 districts in the country are at risk of infection. WHO estimated that about 50% of the districts have been found to be endemic [9]. About one million people carrying microfilariasis (mf) and half a million cases with clinical manifestations are found LF affected areas in Bangladesh [10]. This disease is found almost all the districts in Bangladesh but highly prevalence in Northwestern areas including Thakurgone, Rangpur, Dinajpur, Rajshahi and Nilphamari [11]. It is also endemic in Eastern hilly and forest parts of Bangladesh [12]. The topographical area of both Hobiganj and Moulvibazar districts are same. In case of Satchari tea garden, most of the areas were hilly and the rest area is rather flat and foothill. Sluggish water was found by the side of the flowing streams serve as breeding (reproduction place) source for mosquitoes. Most of the areas of Madonmohonpur tea garden and Patrokhola tea garden are flat. Small portion of these two gardens are hilly and forested. In all these tea gardens there were many drains of fresh water passing through the gardens which was used for tea plants. Such drains also serve as breeding sources of mosquitoes. This area is known for heavy rainfall and numerous "Shed trees" in the tea gardens. Such shaded area is also conducive to some mosquitoes to survive and develop. As LF is transmitted through mosquitoes, so by detection of vector mosquitoes we can control vector mosquitoes in the endemic zone to prevent diseases progression.

II. METHOD AND MATERIALS

This cross-sectional entomological study was carried out in Satchari tea garden of Chunurughat upazilla in Hobiganj district and Patrokhola and Madonmohonpur tea gardens in Kamalganj union of Moulvibazar district. Total duration of this study was six months. Mosquitoes were collected from both Hobiganj & Moulvibazar tea garden. Aspirator and torchlight were used for collecting the mosquitoes (Fig. 1).

Fig. 1. Mosquito collection instruments: paper cup with net, aspirator, and torch light.

Mosquitoes were collected mostly from human living houses. Some were collected from cattle houses. After collection and brought to the laboratory of the department of Biochemistry and Molecular Biology of Dhaka University, The mosquitoes were then identified with Stereoscopic microscope with the help of some mosquito practice book.¹³⁻¹⁶ After identification the mosquitoes were stored in the laboratory for analysis of the density of the vector mosquitoes in the endemic areas and for identifying the vector mosquitoes through molecular method, Polymerase Chain Reaction (PCR).

In case of PCR method, at first deoxyribonucleic acid (DNA) should be extracted from mosquitoes' genome. For extraction of whole DNA from mosquito genome, the materials and method are used according to protocol followed by Linton and his colleagues [17]. This DNA was used as template DNA for PCR. For the identification of species of *Culex quinquefasciatus* the list of reagents and its amount was used and the PCR protocol was followed according to the species-diagnostic polymerase chain reaction assay done by Crabtree [18]. According to this procedure at first primers were prepared which were collected from Commonwealth Scientific Industrial Research Organization (CSIRO), Australia. Sequences of the forward and reverse primers are given below:

Forward- PQIO (19-mer):

5'CCTATGTCCGCGTATACTA 3'

Reverse- CPI6 (29mer):

5'GCGGGTACCATGCTTAAATTTAGGGGGTA 3'

After primer preparation the same way stock solution, working solution of the dNTPs mixture, PCR master mixture, reaction mixture, PCR master mixture for Tag polymerase were prepared. As the PCR were performed in Thermal Cycler. By gradient the annealing temperature of primer was optimized which is 8. The gradient program was designed by keeping 55.1°C in middle. After amplification the PCR products were identified by agarose gel electrophoresis (1.8% agarose). For the determination of the length of the PCR product the marker was used which is 1 kb+ (Invitrogen). After agarose gel electrophoresis, bands for the DNA fragments were visualized by UV trans-illuminator and photographed by gel documentation analysis system.

III. RESULT

A. Mosquito Population Study

In this study a survey has been conducted in Hobiganj and Moulvibazar areas and various types of mosquitoes were collected through the work. The findings are presented in the follow:

TABLE I: MOSQUITO'S SPECIES AND THEIR PROPORTION IN THREE TEA GARDENS OF HOBIGANJ AND MOULVIBAZAR

Sl. No	Name of species	Total Nos.	%	Per-man Hour catch(56 hours)
1	<i>Aedes) lineatopennis</i>	2	0.14	0.04
2	<i>Anopheles barbirostris</i>	3	0.21	0.05
3	<i>An. nigerrimus</i>	153	10.72	2.73
4	<i>An. umbrosus</i>	2	0.14	0.04
5	<i>An. annularis</i>	1	0.07	0.07
6	<i>An. karwari</i>	1	0.07	0.07
6	<i>An. kochi</i>	1	0.07	0.07
8	<i>An. philippinensis</i>	45	3.15	0.80
9	<i>An. pseudojamesi (= ramsayi)</i>	1	0.07	0.07
10	<i>An subpictus</i>	15	1.05	0.27
11	<i>An.vagus</i>	42	2.94	0.75
12	<i>Armigeres kudingensis</i>	55	3.85	0.98
13	<i>Culex annulus</i>	6	0.42	0.11
14	<i>Cx.bitaeniorhynchus</i>	1	0.07	0.07
15	<i>Cx epidesmus</i>	5	0.35	0.09
16	<i>Cx. fuscocephala</i>	92	6.45	1.64
17	<i>Cx. gelidus</i>	139	9.74	2.48
18	<i>Cx hutchinsoni</i>	3	0.21	0.05
19	<i>Cx. mimulus</i>	1	0.07	0.07
20	<i>Cx.quinefasciatus</i>	296	20.74	5.29
21	<i>Cx.sinensis</i>	1	0.07	0.07
22	<i>Cx. tritaeniorhynchus</i>	149h	10.44	2.66
23	<i>Cx.vishnu</i>	345	24.18	6.16
24	<i>Cx.whitmorei</i>	11	0.77	0.19
25	<i>Cx. fragilis</i>	2	0.14	0.04
26	<i>Mansonia annulifera</i>	17	1.19	0.3
27	<i>Ma. Indiana</i>	6	0.42	0.11
28	<i>Ma. uniformis</i>	32	2.24	0.57
Total		1427		25.19

All together 1427 adult female mosquito belonging to 28 species under five genera were collected from Shatchori tea garden in Hobiganj and Patrokhola and Modonmohonpur tea garden in Moulvibazar. In the overall collected mosquitoes in three tea gardens *Culex vishnu* was found in highest amount (24.18%) followed by *Culex quinquefasciatus* (20.74%), *An.nigerrimus* (10.72%), *Cx.geledus* (9.74%) and *Cx.fuscocephala* (9.74%). Besides these other species were collected in small number. Proportion of several of mosquitoes genera in three tea gardens are given below:

From Fig. 2, it is shown that in all tea garden in the study area the number of *Culex* genus mosquitoes was high. Individual in Patrokhola and Modonmohonpur Tea garden, the number of *Culex* genus was high then *Anopheles* and then other genera. But in Shatchori tea garden number of this *Culex* genus was low. Here number of *Anopheles* genus mosquito was high, followed by *Culex armigerus* and then others.

(a)

(b)

(c)

(d)

Fig. 2. Proportion of several mosquitoes genera in different tea garden. (a) Percentage of five genera in all tea garden; (b) Percentage of four mosquito genera in Shatchori tea garden; (c) Percentage of four mosquito genera in Modonmohonpur Tea garden; (d) Percentage of five mosquito genera in Patrokhola tea garden.

B. Detection of Vector Mosquito by Using Polymerase Chain Reaction (PCR):

Whole DNA was extracted from Filarial vector mosquito *Culex quinquefasciatus* and amplified by PCR method using species specific primers,

Forward- PQIO (19-mer):
5'CCTATGTCCGCGTATACTA 3'
Reverse- CPI6 (29mer):

5'GCGGGTACCATGCTTAAATTTAGGGGGTA 3'

which was amplified the 28S ribosomal gene of these mosquitoes. These primers are specific for this 28S ribosomal gene of this *Cx. quinquefasciatus* species. After amplification the amplified product was identified by agarose gel electrophoresis. After run through the gel, the gel was then stained with Ethidium Bromide. Then the band size which is 698 of the amplified products was observed under UV light. The result has been shown in Fig. 3.

Fig. 3. Agarose gel electrophoresis of the PCR product of *Cx. quinquefasciatus* mosquito DNA.

Through this amplification program it can be ascertained that mosquito is truly the *Cx. quinquefasciatus* which is the only filarial vector in Bangladesh. These mosquitoes were collected from three tea gardens in both Hobiganj and Moulvibazar.

IV. DISCUSSION

Bangladesh like other tropical countries is a breed house of different types of mosquitoes. Diseases like Filariasis, Malaria, Japanese encephalitis and Dengue are rampant in our country. Due to poor socioeconomic condition large section of our population has to co-inhabit with mosquitoes. As a result, the above mentioned mosquitoes born diseases are endemic in this country. Eradication of mosquitoes under present socioeconomic graphical status is not possible. However, development of quick diagnostic process to detect and control it may be possible in near future. In this context it is essential to understand the various species of mosquitoes and specially those which are vectors for above diseases, such as Filariasis. With this idea in mind a survey of mosquito population and their characterization was undertaken. Habiganj and Moulvibazar area was selected in the first place to have a preliminary idea about vector mosquito population of filariasis and to identify them in molecular method (PCR).

Overall, 1427 mosquitoes under 28 species were collected from three tea gardens in Habiganj and Moulvibazar. Among the collected mosquitoes three out of thirteen were *Anopheles* mosquitoes which were malaria vectors (*An. philippinensis*, *An. Annularis* and *An. Vagus*). Among these malaria vectors *An. philippinensis* is the primary vector found in high amount (3.15%) and rest of two are secondary malarial vector. Among these 13 types of collected mosquitoes the *Cx. Quinquefasciatus* which is recognized as confirmed Bancroftian filariasis vector in Bangladesh 15 was found in significant amount (20.18%). It was found in highest number in both Patrokhola (24.0%) and Modonmohonpur

(24.04%) tea gardens but insignificant (2.8%) in Shatchori tea garden in Hobiganj. Because this *Cx. Quinquefasciatus* species reproduce in steady and polluted water and maximum places of labours houses in patrokhola and Modonmohonpur tea gardens were flat. Many holes were available, and some drains were passed beside the houses of the labours where steady and polluted water was stored. This polluted and steady water is suitable for breeding of these mosquitoes. For this reason, this mosquito was found in these two gardens. As maximum areas of Shatchori tea garden is hilly so this mosquito cannot breed here. Prevalence of a disease in a certain area depends on the presence of respective vector mosquitoes in that area. So, presence of large number of this species in this area it assumed that transmission of LF is continuing over this area.

Taxonomically vector mosquito can identify but it is not reliable because the closely related grouping mosquitoes cannot be identified with certainly. Molecular method i.e. PCR is very sensitive method to identify the any type of animal or parasitic genome. For this purpose, vector mosquitoes of various diseases can be identified precisely by this method.

Using the species' specific primer of the *Culex quinquefasciatus* the confirm vector of Bancroftian filariasis in Bangladesh was identified through this method.⁷ By this method the vector of other diseases can be perfectly identified using their species' specific primer. As the endemicity of a disease in a certain area is related to the prevalence of its vector in that area, so identifying a specific vector we can ascertain which disease is transmitted over that area. This process of identification of vector mosquito may help eradication of the diseases at early stage of that vector related disease. It is very cost effective and very helpful technique for developing country like Bangladesh to eradicate vector born diseases.

V. CONCLUSION

In this study we observed that Filariasis is endemic in certain areas of Bangladesh. These disabling diseases are affecting down the potentialities of low incoming group of people. Molecular marker may be very effective for rapid identification of the disease. And this may be a very helpful technique to eradicate the vector born disease like Filariasis in the very beginning of diseases progression.

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