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Monitoring Lymphatic Filariasis Interventions Through Adult Mosquito PCR Sampling in South Sumatra Province, Indonesia

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ABSTRACT
Monitoring and evaluation are essential to the successful implementation of mass drug administration programs for lymphatic filariasis elimination. PCR-based methods are preferred over classical dissections with the protocol of one L3 *Brugia malayi* larva in a pool of mosquitoes. We analysed the partial HCL gene nucleotide sequence of the parasite inside the vector. Samples were collected with the methods of human landing, pyrethrum spray, and light traps catch, conducted concurrently at sites in an LF endemic Banyuasin district in South Sumatra. The DNA extraction method was DNAeasy Tissue kit (Quaigen Inc) were used on pools of 10 mosquitoes each. These were used as template DNA in PCR to amplify *B. malayi* sequences and thus sequenced. Our data showed a sequence of the filarial worm in *Mansonia annulifera* and *Culex quinquefasciatus*. To the best of our knowledge, this study is the first report of HLC gene nucleotide sequences from the species *Cx. quinquefasciatus*.

Keywords: *elephantiasis,* Brugia malayi, Culex quinquefasciatus, *HLC gene, surveillance*

1. INTRODUCTION

Lymphatic filariasis (LF) is a vector-borne parasitic disease where currently 856 million people in 52 countries around the world live in risk areas [1]. It is estimated that 91% of LF cases are caused by *Wuchereria bancrofti* while *Brugia malayi* and *B. timori* infections account for the remaining 9% [2]. The parasites are transmitted to humans via mosquitoes from the *Anopheles, Aedes, Culex,* and *Mansonia* genera [3]. Monitoring and evaluation are essential to the successful implementation of mass drug administration programs for LF elimination [4].

Banyuasin has a high rate of endemicity with an average microfilarial rate of 2.02%. It was one of the 142 provinces out of 173 provincials with chronic filariasis cases in Indonesia. The Mass Drug Administration (MDA) programs by the Indonesian official showed a high microfilaria rate which is 0.93% despite the third years' program. Figure 1 presents the case of chronic elephantiasis who suffered from the *B. malayi* infection. Geographically, Banyuasin is located in a lowland filled with swamps, coastlines, rice paddies, and plantation fields, where a mosquito has its ideal breeding ground. Mosquitoes can be used as specimen albite the thick blood smears to check for the parasite that infects humans.

Monitoring transmission using large numbers of mosquito vectors pools was a sensitive method for detecting filarial worm [5]. Effective monitoring to assess the program administered should include less invasive and practical sampling, that the molecular assays on filarial vector have promises [6]. PCR-based methods are preferred over classical dissections since superiority in sensitiveness [7]. The purpose of this study was to analyse the PCR method to detect the filariasis parasite in the mosquitoes as the specimens tested to assess the successful elimination filarial program through the mass drug administration program in Banyuasin district Indonesia.

1.1. Materials and Methods

This experimental analytic with a cross-sectional study design was conducted in April 2017 to May 2018 at the village of Sedang regency of Banyuasin South Sumatra. The samples were mosquitoes' head that potentially contains the L3 larvae of nematode *Brugia malayi*. After the morphological key examination, the specimens were further test with the molecular examination. The positive result amplicons were sequenced and aligned with Filaria nucleotide in Genbank data. Ethical approval obtained from the Ethics Committee of the Faculty of Medicine,

Sriwijaya University, Palembang, South Sumatra, Indonesia.

1.1.1. Mosquito collection.

The research was conducted in Sedang Village which located in Suak Tapeh District in Banyuasin Regency, South Sumatra-Indonesia from April 2017 until May 2018. The research area has the coordinate of 2^0 49.395' of south latitude and 104^0 25.381' east longitude with an altitude of 10 meters above the sea. The studied area has tropical weather with an average temperature of 26-28ºC and humidity ranging from 89% to 92% [8].

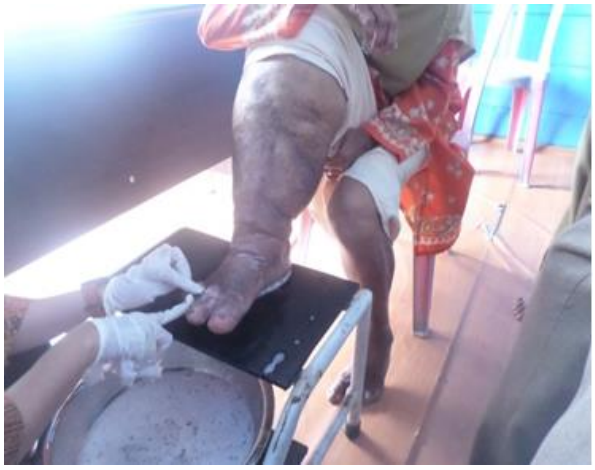

Figure 1 The patient of elephantiasis from the Banyuasin district was prepared to be taken a serial blood test to monitor the presence of the filaria parasite

The trapping process was conducted by the human landing collection (HLC) method performed indoor and outdoor for 24 hours from 18.00 until 17.00 the next day. Six teams were divided into three teams collecting indoor mosquitoes and the next three teams outdoor. The team also collected the mosquitoes rested at wall shelters (rested only) using aspirator for 40 minutes/hour and 10 minutes/hour,

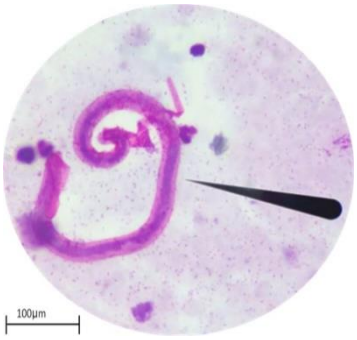

Figure 2 The microfilariae of *Brugia malayi* from the blood test was stained with haematoxylin, take at night to parasite circulate during the nocturnal periodicity

indoor and outdoor respectively. Figure 2 showed the microfilariae from the blood test.

All the collected-mosquitoes were further identified using the Rampa and Wharton identification book and carefully counted [9, 10] as a function of species. Figure 3 showed the mosquito collection methods and the mosquitoes collected. Ethical approval obtained from the Ethics Committee of the Faculty of Medicine, Sriwijaya University, Palembang (Ethical Access Certificate No. 522 / kepkrsmhfkunsri / 2016).

Figure 3 The vector specimens of male and female mosquitoes. Insert: the trapping methods that use human as bait, to confirm that the mosquito was anthropophilic and vector of disease transmission

1.1.2. Polymerase Chain Reaction

The molecular examination for PCR consisted of extraction and amplification steps. DNA isolation was applying the mosquitoes head part of the *Mansonia* spp., *Culex* sp., and other trapped mosquitoes [11]. The extraction examination was performed in the Institute of Eijkman Molecular Centre Jakarta. The isolation of *Brugia malayi* was using the Qiagen Kit catalogue number 69504. Twenty-five mg sample kept in 1,5 ml microtube was smashed with pastel and added with 180 µl buffer ATL and 20µl proteinase K. After vortexed, the sample was incubated 56ºC until lysed. Thus 200µl Buffer AL and 200µl ethanol (96-100%) was added and vortexed respectively. Followed by the spinning period with Dneasy Mini Spin [12].

The amplification of HLA gene locus was performed using the forward Filaria-F (5'-GCGCATAAAT TCATCAGC-3') and the reverse -R (5'-GCGCAAAAC TTAATTACAAAAGC-3'). The PCR temperature and the master mixes were according to the published paper [13]. The amplicon later electrophoresed 80 volts for 40 minutes. The gel was 2% agarose TAE with ethidium bromide and read after the DNA ladder addition under ultraviolet light using Gel doc. The PCR results have a positive result if there is a band at 326 bp as *B. malayi*.

1.1.3. Sequence comparison and phylogenetic analysis.

Samples with a positive band's weight were further sequenced. PCR products of 20 µL were sent along with the primer to the First Base company in Malaysia for sequencing. Thus, the results obtained were analyzed with Blast NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

Phylogenetic trees were generated from the closest similarity to the sequenced samples. The query cover (percentage of the same base length between the sample sequence and the database) and the percent identity (percentage of similarity in the reading of sample sequences with sequences in the database) will determine the closest similarity to the sequenced samples. The organism's name and the accession number (the reference number of the organism's database number) were rooted by the website machine. Sequence analysis and phylogenetic tree of the sample were provided from the NCBI gene bank website.

1.2. Our Contribution

This paper presents the successful alternative monitoring of filaria and also first reports of new potential filarial vectors. HLA gene locus amplification from the vector was able to be detected among pools of mosquito's genus. The genus of *Culex* and *Mansonia* molecularly tested and aligned to *Brugia malayi* sequence nucleotide in Gen Bank. The *Culex* species which sequenced positive with the parasite was not known before to harbour the parasite and become the vector of disease. The molecular assays proved a valid tool for monitoring the transmission of disease, and also suggest a new approach to tackle the disease elimination since new species has particular bionomic than others.

1.3. Paper Structure

The rest of the paper is organized as follows. The first section introduces the mosquito trapping and collection, which used the specimens for the material to be assessed with the molecular method. The second section described of the PCR process consists of extraction and amplification which search for the positive correct band weight using the agarose gel visualization. The third section presents the sequence analysis and phylogenetic alignment of vector material. The sequenced data further analyze with the parasite nucleotide that stored in the GenBank data. The last section concludes the findings.

2. RESULTS AND DISCUSSION

2.1. Specimen Results

During the research period, there are 7908 mosquitoes collected which consists of 13 genera including *Mansonia, Culex, Aedes, Anopheles, Coquilettidia, Topomyia, Armigeres, Triptoides, Miomyia, Malaya, Uranataenia, Hodgesia* and *Urotonia* [11]. Most genera are commonly found in Tropical countries such as Indonesia, Malaysia, *etc.*

Further from 13 genera, all the obtained mosquitoes are analysed and divided into 40 species. Moreover, the most dominant diversity is coming from *Culex* spp. which have 12 species, followed by *Aedes* (9 species), *Mansonia* (6 species), *Anopheles* (3 species), *Coquilettidia* (2 species), and 1 species for *Armigeres, Triptoides, Malaya, Urotonia, Uranataenia, Topomyia, Coquilettidia, Hodgesia, Topomyia,* and *Miomyia*. However, *Mansonia* spp. is found as the highest collected mosquitoes where 4.448 *Mansonia* spp (56.30%) have successfully collected and identified during the research period. On the other hand, *Culex* spp. as the highest species diversity has the second abundance of collected mosquitoes which consists of 1.843 mosquitoes (23.33%).

2.2. PCR Results

There were two positives pools, the *Mansonia annulifera (I4)* and *Culex quinquefasciatus* (I8) mosquitoes. Positive samples resulted in the appearance of the white band in 32*6* basepairs (Figure 4). Negative samples showed no white band or white band in the wrong band weight. This occurs due to the primers also attached to other sites besides the HLA gene. Therefore, the false positive samples showed the different sizes of the white band. True positive samples were furthered test for sequence analysis.

Figure 4 Agarose gel with ethidium bromide electrophoresis UV visualization of pools of samples. Positive PCR amplicons of *Brugia malayi* were at 324bp. Caption: (I4) basepair; (I8) samples' number. Insert: the false-positive result of the PCR process, where the white band was in the wrong basepair size

2.3. Sequence Result and Phylogenetic Tree

The sequences process should be first assessed for the quality of chromatoform. Figure 5 showed the result of the sequence obtained was good. Thus, the alignment of the samples with the one of *B. malayi* that available online can be applied. The phylogenetic tree of samples I4 and I8 can further be assessed and show high similarity with difference only 0,02%.

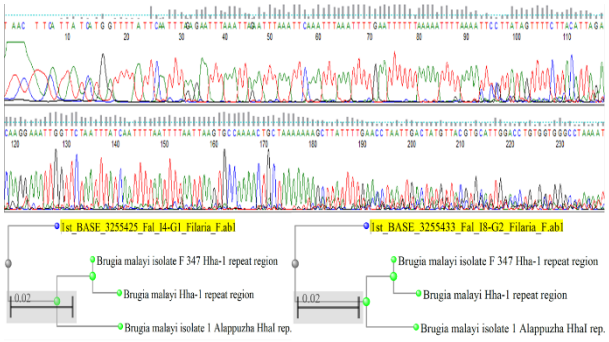

Figure 5 Sequence analysis and phylogenetic tree of the mosquito sample alligned with the NCBI Genbank website showed high similarity to *Brugia malayi*

3. CONCLUSION

Monitoring lymphatic filariasis interventions can be easily done through adult mosquito PCR sampling. To the best of our knowledge, this study is the first report of HLC gene nucleotide sequences from the species *Culex quinquefasciatus*.

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