

Filariasis: Facts and Control Strategies in Indonesia

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Abstract

Lymphatic filariasis is a neglected tropical diseases, which infection occurs when filarial parasites are transmitted to human through mosquitoes' bite. In Indonesia, the infection incidence was increased significantly throughout these recent years, it was reported about 6,998 cases in 2004, and whereas in 2018 there were 12,677 cases. Moreover, Indonesian government has conducted two strategies, firstly by applying MDA to reduce the incident rate (mf rate <1%), and secondly by managing and monitoring chronic filariasis patients. However, there were still limited reported studies on the filariasis vector surveillance and control. In this study, we are focus in urban filariasis with *Culex quinquefasciatus* as the main vector of *Wuchereria bancrofti*. This mosquito was active during night time, at around 10 pm and 2-4 am, more likely active outdoor rather than indoor, and caught mostly during biting rather than resting activities. Their breeding places were mostly in stagnant and polluted water. In some endemic areas showed the high genetic variation among these mosquitos and defend proteins were detected in positive mosquitos. These proteins present in mosquitos to fight back the filarial parasites.

Keywords: *Culex quinquefasciatus*, Genetic Variation, Defense Protein, Lymphatic Filariasis, *Wuchereria bancrofti*

Introduction

Vector-borne mosquito diseases are still as major public health problem in the world, such as lymphatic filariasis (LF). There are three species of nematodes (Family: Filaroidea) which are the causative agents of this disease, namely *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. This disease cause changes in the damage to the lymphatic system and can lead to the enlargement in abnormality of body parts, known as elephantiasis. Then, it will causing limitation of motion and other disability, pain, economical-loss and social stigma [1, 2].

In 2000 more than 120 million people in 73 countries from Asia, Africa, the Western Pacific, parts of the Caribbean and South America (Brazil, Haiti, Guyana) were infected and in which 83 million of them have elephantiasis [2, 3]. Moreover, there are about 1,3 billion people live in high risk endemic areas [4]. WHO declare the Global Programmed to Eliminate Lymphatic Filariasis (GPELF) in 2000, and achieving elimination by 2020 as the target date. One of LF elimination efforts is by stopping the spread of infection through preventive chemotherapy with Mass Drug Administration (MDA). The MDA is done by administering an annual dose of medicine to the risk population [2]. This program was deemed effective and success in some countries, as reducing the number of cases and after

surveillance there was no new transmission [4]. It was reported that in 2019 globally 597 million people no longer require the preventive chemotherapy [2].

In Indonesia, the LF cases was increased significantly by years. As reported, there is 6,998 cases in 2004 and become 12,677 in 2017 [5]. Twenty-eight out of 34 provinces in Indonesia were declared as filariasis endemic areas, which 236 of city/regency have the case of filariasis and were LF endemic areas [6].

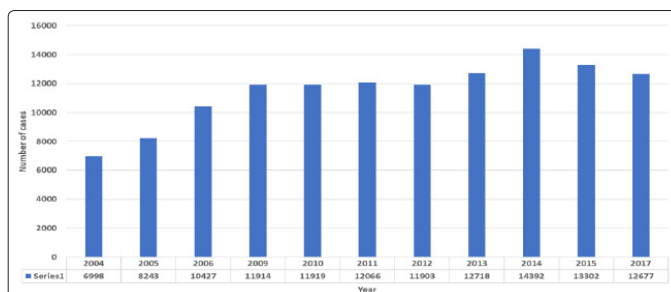


Figure 1: Number of Filaria cases in Indonesia from 2004-2018 [5].

The government actively conduct elimination filariasis programs which is MDA, especially to the risk communities in endemic areas and provide basic package of care to manage the chronic cases patients. However, there were some obstacles in implementing

the MDA program, which are the large number of endemic areas, the geographic and topographic differences, and the limitation of community awareness for LF disease. Moreover, there was lack of information for the vector surveillance and control for LF. The purpose of this report was to convey the latest development handling filariasis in Indonesia and to examine the distribution, behavior, genetic variation, defense proteins present in *Cx. quinquefasciatus* mosquitoes from filariasis endemic areas.

Material and Method

The latest sources of LF control strategies in Indonesia was acquired from the National Seminar on Mosquito Control Day in August, 2019 in Semarang Indonesia. In this study, the distribution, behavior, genetic variation, and identification of defense proteins of mosquitoes were studied in four LF endemic areas in Central Java, which are: Pekalongan Regency, Pekalongan City, Semarang Regency, and Semarang City. This study has been followed strict research ethics guidance and obtained ethical clearance from the Ethic Commission, Number: REF: KE/FK/0612/EC/2017.

Mosquitoes were collected in hourly interval by human landing method and insect net, from 6 pm to 6 am in the next day. The number of mosquitoes were calculated, and separated by their activities indoor or outdoor and for biting or resting. Moreover, the Random Analysis Polymorphic DNA (RAPD-DNA) was used for the genetic variation study [7-9]. The mosquito DNA was isolated by using DNA-extraction kit from gSYNCTM (Geneaid, Cat. No. GS 100, PT. Genetika Science Indonesia), with the 10-mer RAPD primers were OPA11, OPA 12, OPA15, OPA16, and OPA20 (1st base). The amplified samples were then run into 2% agarose gel for 45 minutes. Isolation of mosquito's protein profile following the method of Munoz et al. [10], as well running into SDS-PAGE on 1.8% agarose gel [11].

Number of mosquitoes and their activities from the four areas were analyzed by Kruskal-Wallis test. Descriptive analysis were used for the analysis of mosquito genetic variation and the protein profile.

Results and Discussion

Since 2000, it was about 7.7 billion treatments has been done to stop the LF spreading. It was success activities, because it was said that 597 million people were no longer to take the preventive chemotherapy. However, there were, 947 million people in 54 countries worldwide still remain threatened by LF and require preventive chemotherapy. By 2018 it was reported that 893 million people in 49 countries required preventive chemotherapy to stop the spread of infection [2].

In Indonesia the preventive chemotherapy has also been done, especially in 236 regency/city endemic areas. By 2018 5-years MDA cycle activity was finished and succeed in 105 regency/city. The filariasis elimination certificate has been also given to 22 regency/city, and the rest of 83 regency/city still under surveillance post 5-year MDA cycle. Nevertheless, there were still 131 regency/city still carrying out of the filariasis MDA. There are two pillars for the achieving filariasis elimination as the Ministry of Health regulation (number 94, year 2014). It was stated in the regulation that the regency/city mapping for the microfilarial rate (mf-rate) is higher than $\geq 1\%$, the preventive chemotherapy should be carried out and while the chronic cases management will be given as disabilities care a package to minimize suffering and to promote a better quality

of life will be given to those who impacted by severe LF.

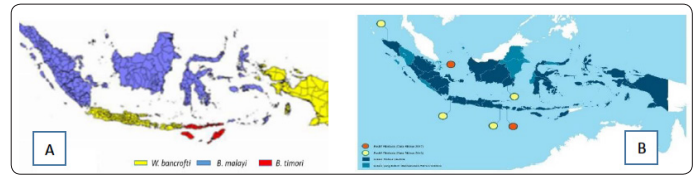


Figure 2: (A) Distribution of filaria cases by the species of nematodes in Indonesia [12]; (B) Distribution of vector with positive filaria in Indonesia [13]

Indonesia, the three causative of LF were founded and there were presented in Figure 2A, Furthermore the newest reported from Indonesian vector research from 2016-2018 showed that there was new finding of the positive mosquitoes with the infective filaria larva in the areas with negative case reported before (Figure 2B) [13].

Mosquito is worldwide distributed in tropics and subtropic region, closely to human habitation, and about 3,400 species has identified [3, 14, 15]. Up to 1981, 18 genera, within 456 mosquito species has been identified in Indonesia [13]. Based on different geographic and topographic condition, in Indonesia there were about 23 of mosquito species from 5 genera confirmed as vector of the filarial parasites, they were: *Mansonia*, *Aedes*, *Culex*, *Anopheles*, and *Armigeres* [14, 16].

This study was focusing on the urban filariasis with *W. bancrofti* as the causative agent and *Cx. quinquefasciatus* as the main vector [17]. The adult worms live in afferent lymphatic vessels and the microfilaria (mf) periodically circulate in the human peripheral blood [1]. For this study, there were 6 observation areas at the 4 endemic regency/city in Central Java. In these urban and semi urban areas the mf transmission was periodically nocturnal.

The observation showed that *Cx. quinquefasciatus* mosquitoes could be found with different biting and resting activities, in both time period and individual number for each areas were also recorded. There was significantly different ($\alpha, p < 0.05$) of the mean mosquito indoor biting and resting activities, also the outdoor biting and resting activities from the four areas of collection. The highest number of individual mosquitoes was found in Semarang City (266), followed by Pekalongan Regency (108), Pekalongan City (19), and Semarang Regency (12).

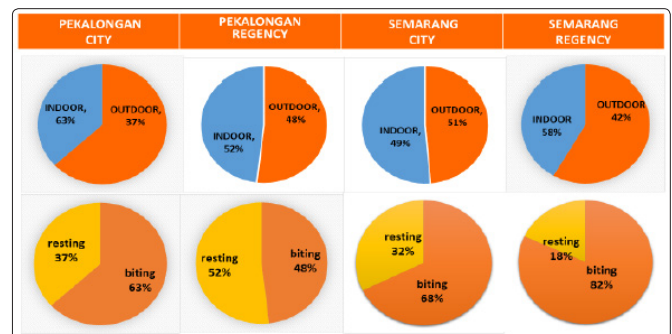


Figure 3: Proportion of indoor and outdoor for biting dan resting activities of *Cx. quinquefasciatus*

Overall the *Cx. quinquefasciatus* show patterns of biting and resting activities that vary throughout the night and tend to be biting outdoor rather than indoors, and prefer to rest outside the home. Citizen's

habits of slow sleep and activities outside the home in a period of 10 pm to 3 am are likely to be at risk for getting the disease. Windiastuti et al. stated that the habit of being outside the home is one of the risk factors for contracting filariasis with a probability up to 99.9% [18].

The peak of indoor biting activity was between 10 pm to midnight, and still high up to 02-03 am. The peak of outdoor biting was between 11 pm-3 am in Semarang City and Pekalongan Regency respectively (Figure 4) This is still alliance with report Mak and Dennis and Sutanto et al. stated that the mosquito biting activity was around midnight [14, 19]. Sacks said that the peak period of mosquito activity is positively related to the activity of microfilaria (mf) in the patient's peripheral blood between midnight and 2 am [20]. The presence of abundant mf in the peripheral blood at night is stated to be related to the presence of the melatonin hormone which is excreted at night. This hormone will increase at night and reach its peak at 10 pm which will further decrease sharply after 2 o'clock in the morning [20].

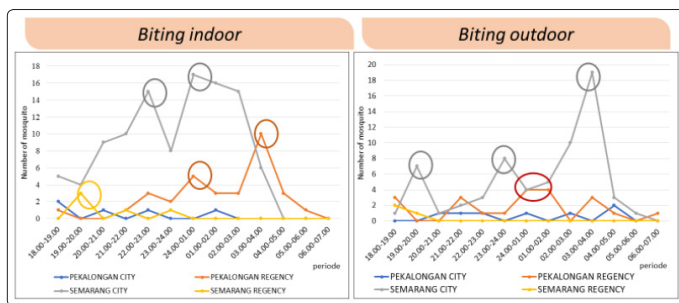


Figure 4: Indoor and outdoor biting activities of *Cx. quinquefasciatus* from the site of mosquito collection

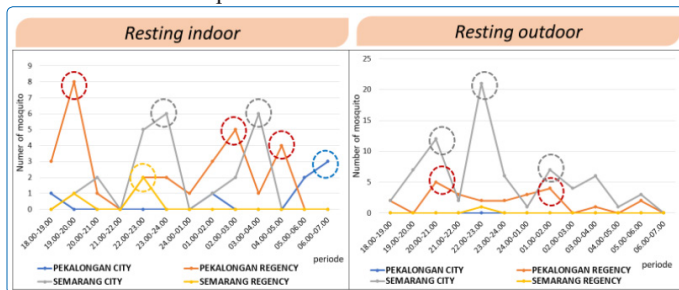


Figure 5: Indoor and outdoor resting activities of *Cx. quinquefasciatus* from the site of mosquito collection

The pattern of indoor and outdoor resting activities varied by mosquitoes from four locations. As presented in Figure 5, mosquitoes showed an indoor resting pattern with peaks at 07 pm, 10 pm, 11 pm, 02am-04 am, and 06 am WIB, which is likely related to flying mosquitoes from the breeding place to the food source. Bram stated that the exodus of adult mosquitoes from their breeding sites was at dusk and reached a peak at 08 pm [21].

Gillot and Manguin et al. said that environmental changes affect insect activities which in turn will have an impact on their diversity and distribution. In this study the environmental parameters, such as temperature, humidity and wind velocity show a significant effect on mosquito activities [3, 22]. The measured air temperature ranges from 25-31 °C, humidity ranges from 61-98%, and measured of wind velocity range from 0-25.8 m/h. In this study was observed that the increase of temperature and humidity caused increasing of

mosquito activity, while the wind velocity have negatively affected in biting activity, but had a positive effect on resting activity as reported by Astuti [17]. Manguin et al. said that physical factors and changes such as temperature and humidity are very influential on the vector capacity, and at higher temperature and sufficient humidity will increase the development of pathogens in the vector [23]. Moreover, the rising temperatures cause dissolved oxygen levels in the breeding places so that it will modify the activity of insect in the success rate of their life [22].

Eighty one mosquitoes from the four site of collection were studied for the genetic variation. A total of 945 fragment as genetic characters were identified and about 882 of the *Cx. quinquefasciatus* characters were genetically areas specific [17]. As Qun-Liu et al. stated that at a level of 50% of polymorphism it can be stated that the species has a high diversity, the four site mosquitoes collection also showed high polymorphism [24]. The polymorphic levels of mosquitoes from Pekalongan City and Pekalongan Regency was less than 12% in similarity, while mosquitoes from Semarang City and Semarang Regency with a similarity level less than 2%. It was said that in Pekalongan and Semarang, both showed high genetic variation, even with polymorphism reaching 100% [17]. Frankham et al. said that the genetic character differences are caused by the spread of mosquitoes that are wide and fast, mingling of mosquitoes from different breeding sites will cause gene flow in certain location to be high so as to bring more diverse character [25].

Study on protein profiles showed that there were proteins responsible for the defense mechanism of mosquito to filarial worm which detected as distinct band at 4.5 – 104.2 kDa with SDS PAGE and about 4.53 – 12.07 kDa by using Liquid Chromatography Mass Spectrometry (LCMS) [26]. Sun et al. Vizioli et al. and Kumar and Paily stated that there was specific protein as detected from *Cx. quinquefasciatus* mosquito to defend against *W. bancrofti* infection [12, 27-30] that is were cecropin with the molecule weight 3-4 kDa, defensin at the 7 kDa, and transferrin at the 66 kDa. This study revealed that there was two molecules proteins detected from positive mosquitos at the molecular weight 7 kDa and 66 kDa. It can be stated that these proteins are characteristic of the biological response of *Cx. quinquefasciatus* against *W. bancrofti* infection. This finding indicated that there was filariasis transmission, and the present of proteins in mosquitos with no new human case detected can become an indicator of the successfulness of MDA implementation in endemic areas

The preventive action that can be done by communities, by their own are to keep clean environmental condition for the preventing vector development, such as good drainage system in terms to reduce the mosquito population, secondly to reduce contact with mosquitoes by using mosquito net, natural repellent, etc.

In conclusion there were two strategies to stopping the spread of LF infection, firstly by implementing large-scale multi years treatment, 5 years MDA cycle in endemic areas and by improving the management of chronic cases patients. It was suggested that there should be a continuous monitoring and assistant for health and psychological aspects of the LF patients. Government should make a specialized internship program for LF patients, so that they can earn a living and more independent. The community based education should be done and continuously for the optimization of vector control by especially to raise the awareness of community for

reducing the breeding sites, reducing or no contact with mosquitoes. Research of using natural insecticides that are environmentally friendly should be also encouraged.

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