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Status of Lymphatic Filariasis Transmission after Two Additional Rounds of Filariasis Mass Drug Administration: A Case Study in Pekalongan City, Central Java, Indonesia

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Abstract

BACKGROUND: Lymphatic filariasis (LF) is a neglected tropical disease caused by mosquito-containing filarial worms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The World Health Organization had set up a global program to interrupt transmission with mass drug administration (MDA) and manage morbidity and prevent disability.

AIM: This study aimed to determine the status of LF transmission in Pekalongan City after two additional rounds of MDA. A cross-sectional study was conducted in Pekalongan City, Indonesia.

MATERIALS AND METHODS: Three villages were selected purposively as study sites: free, non-endemic, and endemic of LF as more than 600 finger blood samples were taken from study participants in the night between 21:00 and 01:00. Laboratory testing for microfilaria examination was conducted in Bendan and Bedono Public Health Center Pekalongan, Indonesia. Mosquitoes were collected in three areas of the study site from the middle of the night until the morning. Identification and biomolecular examination of caught mosquitoes used a polymerase chain reaction. Statistical analysis was made using the IBM SPSS Statistics 26 software.

RESULTS: This research found two microfilaria-positive samples from 600 finger blood samples examined (microfilariae rate: 0.33%). There were four positive reactions to filaria. The identified mosquito species consisted of *Culex quinquefasciatus*, *Culex vishnui*, and *Aedes aegypti*. *Aedes aegypti* was only confirmed filaria positive.

CONCLUSION: A non-endemic village was only found one blood sample positive, but blood and mosquito samples positive were found in endemic villages. It seemed that LF transmission was still in running di endemic village in Pekalongan City.

Introduction

Lymphatic filariasis (LF) is a neglected tropical disease caused by mosquito-containing filarial worms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The World Health Organization (WHO) had set up a global program to interrupt transmission with mass drug administration (MDA) and manage morbidity and prevent disability [1]. The global program to eliminate LF is conducted through MDA. The success of MDA depends on coverage and compliance in taking medication of people who live in LF endemic areas [2]. Based on the previous experience, proper MDA dramatically reduced filariasis infection markers. These included microfilaremia, filarial antigenemia, antifilarial antibodies, and parasites in mosquitoes that transmit the infection [3].

Filariasis has been a public health problem in Indonesia for a long time. The WHO has established this disease as a neglected disease that is a public health problem globally. Therefore, a global filariasis elimination program must be achieved in 2020 [4]. Around 94,005 people in the world were reported to be affected by LF in 2019. Indonesia maintained 100% geographical coverage with MDA for the 3rd consecutive year and achieved adequate coverage of 96% [5]. There are 10,861 LF cases in Indonesia, and they spread in 34 provinces, 514 districts, and 236 districts are endemic areas of LF. Central Java is one of Indonesia's provinces that place the seventh rank of LF filariasis cases. There are nine districts of the LF endemic area with 505 LF cases registered. One of them is Pekalongan District [6], [7].

Pekalongan, Indonesia, was known as an endemic area of LF for approximately 30 years. MDA was implemented in the area for 5 years (2011–2015). The treatment coverage (2011–2015) was reported at 89.95% and did not achieve success [8], [9]. Unfortunately, in the pre-transmission assessment survey (TAS) in 2016, 1.45% revealed that the mf rate was still higher than >1% [6], [7], [10]. The program conducted for LF control included finger blood survey, treatment, and case management. It was not conducted regularly but still accidentally. Therefore, two additional MDA rounds (2017–2018) had to be conducted in Pekalongan.

Moreover, no vector surveillance or control was addressed for LF elimination specifically. Other factors contributing to LF transmission were environmental and community behaviors, especially about medication compliance [1], [11]. Pekalongan was a coastal and flooded area. It leads to creating a large number of suitable breeding places for mosquito development [12]. This epidemiological condition was supported by the previous research that it was still found from both positive finger blood survey and entomological survey [10]. Thus, this research was conducted to determine LF transmission status in Pekalongan after two additional rounds of MDA. The research contribution is that monitoring the transmission dynamics during MDA implementation is essential for measuring the progress and defining the endpoint of MDA.

Materials and Methods

Study design

A cross-sectional study was conducted in Pekalongan City from September to December 2020. Pekalongan, a *Wuchereria bancrofti* endemic area, is a coastal area. LF is transmitted by *Culex* sp. Research locations in three villages were selected purposively as a study sites classified into three areas: free, non-endemic, and endemic of LF (Figure 1). This study obtained ethical approval from the Ethical Committee of Public Health Diponegoro University, no. 141/EA/KEPK-FKM/2020, dated June 30, 2020.

Figure 1: Map of the study area

Sample

In this research, 250 blood samples were taken from the free village of LF, 250 samples from the non-endemic village, and 100 samples from the endemic village of LF. Calculation of the sample size in this study was done using the CSurvey 2.0 application. The sample calculation in the CSurvey 2.0 application gave a large sample size for the two-stage cluster sampling technique, namely selecting a cluster from a sampling unit in the first stage and selecting subjects from each cluster in the second stage. The selection of clusters is carried out by processing in a computer program using the WHO guidelines for the probability proportional to size cluster sampling technique. Then, a simple random sampling technique selected the selection of subjects in each cluster for each neighborhood unit.

Laboratory blood examination

Six hundred finger blood samples were taken from the study participants in the night between 21:00 and 01:00. Laboratory testing for microfilaria examination was conducted in Bendan and Bedono Public Health Center in Pekalongan City. First, the examination of microfilariae was carried out using Giemsa's stain for 30 min. Then, a microscopic examination was performed at a magnification of ×100. The Giemsa solution is a solution used for staining capillary blood preparations made from a pH-7 buffer fluid. The pH-7 buffer liquid is made by dissolving one tablet of buffer forte into 1000 ml of clear and clean water. This buffer fluid can also be replaced with mineral water, which has a pH of 7. The Giemsa solution is made by dissolving Giemsa liquid with the pH-7 buffer liquid at a ratio of 1:20.

Before staining, the capillary blood preparations are hemolysis with water for a few minutes until the red color disappears. It was then fixed using absolute methanol for 1–2 min. Next, the capillary blood specimens were stained by dropping the Giemsa solution until all preparation surfaces were immersed in Giemsa solution (approximately 20 drops) and allowed to stand for 30 min. Then, the capillary blood was rinsed with clean water and dried at room temperature. After drying, the capillary blood sediment was arranged and stored in a slide box. Finally, the stained capillary blood was examined microscopically at 100× magnification using oil immersion. Filariasis examination is based on the finding of microfilariae in the peripheral blood smear.

Polymerase chain reaction test for filarial identification

Four thousand five hundred and forty-seven mosquitoes were collected in three areas of the study site from the middle of the night until morning between 21:00 and 06:00. The manual aspirator was used as a tool for mosquito catching. Caught mosquitoes were transferred from the aspirator to labeled paper cups covered with a netting material and transported to the laboratory for morphology identification and biomolecular examination using polymerase chain reaction (PCR). PCR reaction was conducted at the Health Research Institute Laboratory, Ministry of Health Banjarnegara.

Only female mosquitoes were examined to determine whether they contained positive filaria deoxyribonucleic acid (DNA). DNA was extracted using an Extraction Kit (IQ Plus™ Extraction Kit). DNA extraction was carried out simultaneously using the spin column technique (simultaneous) and used in the amplification process. Mosquito samples were put in a 1.5 ml microtube, and 500 µl of solution-1 (lysis solution) was added; the mixture was then crushed with a pestle. After all the test materials were lysed or destroyed, 500 µl of solution-2 (a solution containing alcohol as a DNA binding) was added, mixed well, and precipitated (spun) for 1 min to separate protein and DNA. A total of 500 µl of a supernatant-containing DNA was transferred to the spin column tube and then deposited (spun) for 1min. Then, the liquid in the container tube was removed while the nucleic acid was at the bottom of the spin column. The rinsing process was added to solution-2 again, as much as 500 µl on the spin column. It was deposited for 3 min, and the solution that was accommodated in the tube was then discarded. The spin column (containing DNA nucleic acid) was transferred to 1.5 ml of a new microtube, and 200 µl of solvent/nucleic acid elution (for tissue samples) was added and then precipitated (spun) for 1 min. Furthermore, the DNA obtained amplifies the third instar larvae of filarial worms. DNA amplification of third instar larvae using IQ Plus™ DNA for third instar larvae of filarial worm kit was carried out.

Statistical analysis

We used the IBM SPSS ver. 26, IBM Corp, Armonk, New York, USA software for statistical analysis. Data are sorted according to the study area. The finger blood and mosquito results for the mf rate and filarial PCR reaction sample were compared using frequency distributions. The map was created on Google Maps using a remote sensing image.

Results and Discussion

This research was conducted to determine Pekalongan, Central Java, Indonesia (Figure 1). Data collection was done from September to December 2020. Research activities include finger blood sampling, laboratory examination for microfilaria determination, mosquito catching, mosquito identification, and laboratory examination to determine filaria DNA.

This research found that two blood samples (microfilaria rate: 0.33% overall) were positive from the 600 finger blood samples. In addition, Medono village had a 0.40% microfilaria rate (mf rate), and Jenggot village had a 1.00% mf rate. Table 1 describes the result of the finger blood laboratory examination for determining microfilaria.

Table 1: Results of 600 finger blood laboratory examination by study site

Serial number	Study site	Number of blood samples examined	Number of positive samples	Microfilaria rate (%)
1	Bendan village	250	0	0.00
2	Medono village	250	1	0.40
3	Jenggot village	100	1	1.00
	Total	600	2	0.33

Four thousand five hundred and forty-seven mosquitoes had been caught, and 427 (20.39%) of them were female. When the PCR technique was used, of 119 mosquito pools (each with a maximum of 10 mosquitoes) tested, four were positive filaria DNA (positive rate: 3.36%). All mosquitoes that were positive filaria DNA were from Jenggot Village (positive rate: 10.26%). Detailed data are presented in Table 2.

Table 2: Results of mosquito laboratory examination using polymerase chain reaction

Serial number	Study site (village)	Number of mosquitoes caught	Number of female mosquitoes tested using PCR	Number of PCR reactions	Number of positive reactions	Positive rate (%)
1	Bendan	1,959	377	40	0	0.00
2	Medono	2,340	382	40	0	0.00
3	Jenggot	248	168	39	4	10.26
	Total	4,547	927	119	4	3.36

PCR: Polymerase chain reaction

Before conducting an examination using PCR, female mosquitoes were identified based on species. Three species were found in the study site: *Culex quinquefasciatus*, *Culex vishnui*, and *Aedes aegypti*. Data are described in detail in Table 3.

Table 3: Results of mosquito identification and its polymerase chain reaction examination

Serial number	Study site (village)	Mosquito species	Number of female mosquitoes tested using PCR	Positive filaria DNA
1	Bendan	*C. quinquefasciatus*	377	0
2	Medono	*C. quinquefasciatus*	382	0
3	Jenggot	*C. quinquefasciatus*	159	0
		C. vishnui	3	0
		A. aegypti	4	4
	Total		927	4

PCR: Polymerase chain reaction, *C. quinquefasciatus*: *Culex quinquefasciatus*, *C. vishnui*: *Culex vishnui*, *A. aegypti*: *Aedes aegypti*

As additional information that Medono village was non-endemic of LF. This village had a history of LF existence in the past period, although not every year. On the other hand, Jenggot village was endemic of LF, and there were LF cases every year. Although MDA had implemented additional two cycles in all villages in Pekalongan City, this research still found mf positive based on finger blood examination. It means that implementing two additional rounds of MDA did not ensure that this area was free from LF cases. However, it could reduce the mf rate to 1%. As previous research stated, the epidemiological situation after eight rounds of MDA led to a reduction of transmission to 0.1% in the study area [13].

Many factors contributed to the existence of LF cases in the study area. One of them was community participation in supporting LF control by consuming filarial medicine. The quality of LF control depends on not only MDA coverage but also compliance in consuming the medicine. There are many factors and specific experiences associated with compliance or non-compliance. Specifically, individuals need to trust the government, health workers, and the person delivering drugs [8], [14]. Elimination programs should ensure that trust elements are built into campaigns to engage communities effectively [2]. Using the health belief model approach, several aspects of medication adherence are perceived susceptibility, perceived severity, perceived benefits, and perceived barriers [15]. Previous research has also demonstrated various problems during the MDA program in Pekalongan. The problems are inaccurate population data, refusal to take medication due to side effects, adherence to taking medication, health–illness perception, and delays in drug distribution, which resulted in limited time to pack drugs according to age groups [8], [14], [16]. The role of elimination officers was essential in increasing community knowledge about the MDA program and the benefit of controlling the LF disease.

The identification result indicated that *Culex quinquefasciatus* was the dominant mosquito in the study site. This research found that *Aedes aegypti* was the only mosquito that was positive filaria DNA. The *Aedes aegypti*, which had positive filaria DNA, was caught from four catching points in Jenggot village. Therefore, this finding can ensure that *Aedes aegypti* takes a prominent role of LF transmission in Jenggot village, Pekalongan City. Unlike previous studies that confirmed that *Culex quinquefasciatus* was the only mosquito as the vector of LF [17]. A previous study found mosquito-containing mf positive in Pabean Village, Pekalongan City, one village near this study site. Of the 16,767 dissected mosquitoes, three were positive of microfilaria larvae-3 (positive rate: 0.02%). It had been identified that *Culex quinquefasciatus* was the primary vector of LF transmission [18]. Research conducted in Hulu Sungai Utara District found one species causing LF of the 311 *Mansonia uniformis* in 13 microtubes tested. One tube was positive for *Wuchereria bancrofti* infectivity rate of 0.3% [18]. Based on this history, it can be stated that no single vector transmits LF. *Culex quinquefasciatus* and *Aedes aegypti* are two species that had ever been found to be LF vectors in Pekalongan City.

Culex quinquefasciatus and *Aedes aegypti* were urban mosquitoes. Artificial breeding places were created by wastewater mismanagement, resulting from low sanitation systems and industrial pollution. Pekalongan City was characterized as an industrial area, especially batik fabrics. Many fabrics released wastewater into open drainage or irrigation channel. This environment was a very suitable breeding place for urban mosquitoes such as the *Culex* genus. Previous studies have stated that irrigation, mismanagement of wastewater, water storage, and waste buildup lead to increased bite rates. Bite rates cause higher transmission potential and the proportion of vectors that infect or are infected with microfilariae [19], [20], [21].

Mosquito biting activity patterns vary widely. This pattern is caused by the difference in the degree of adaptation of each mosquito species in different environments. The behavior of mosquitoes in finding a host at night is associated with an increase in light intensity, especially in the *Aedes albopictus* mosquito, which is sensitive to dim light. The activity to find the host will stop entirely in total darkness. Besides, the flight behavior of mosquitoes is influenced by the circadian rhythm in the mosquito's body. The presence of light may directly influence mosquitoes' activity at night and indirectly affect the regulatory phase of endogenous rhythms in the mosquito's body. The nighttime activities of *Aedes* spp. are more caused by mosquitoes' intrinsic reaction to light so this behavior can increase disease transmission in both urban and rural areas [22], [23], [24].

Mosquito *Aedes aegypti* is known to have a high vector capacity due to its anthropophilic nature, good domestication, and adaptation to survive in different geographical areas, including Africa, America, Asia, and Europe. *Aedes aegypti* usually prefer to feed on mammalian hosts and would love to bite humans, even in the presence of other hosts (anthropophilic behavior); this behavior, along with many feeding habits and highly domesticated behavior, can make it an efficient vector [25].

Epidemiologically, filariasis can involve many complex factors: filarial worms as disease agents, humans as vectors, physical, biological, and social environmental factors, namely socioeconomic factors and residents' behavior. Apart from reservoirs and vectors, the environment is also essential in the transmission process. The environment can support reservoir and vector survival. The environment is critical in the epidemiology of filariasis, such as the type of filariasis that can be estimated by looking at the environment [25]. The estimated vector capacity is influenced by one environment that affects the relationship between vectors and pathogens to be transmitted [26].

Mosquito control was essential in interrupting LF transmission in an endemic area. This strategy was both feasible and appropriate. However, MDA dramatically reduced all filariasis infection parameters in people, and parasite DNA rates in mosquitoes fell more rapidly [3]. However, the MDA implementation's success could not be guaranteed without supporting vector control activities as a complementary measure in the LF elimination program [27]. Thus, surveillance activities are vital on humans and vectors as LF control in an endemic area. This concept was relevant to the expert statement that surveillance is necessary for regions with a low prevalence that does not require MDA but is proximal to endemic areas due to the risk of introducing infection [28].

Conclusion

After two additional MDA program cycles, this research concluded that LF transmission is still ongoing in Jenggot Village, Pekalongan City. This conclusion was strengthened by laboratory examination of the finger blood test and the mosquito's positive filaria DNA. *Culex quinquefasciatus* is the dominant mosquito caught in the study site, but *Aedes aegypti* was the only mosquito species with positive filaria DNA and played a role in LF transmission. Despite a substantial decline in LF in this study area, a recommendation to stop MDA could not be made because the mf result and the current study methods did not follow the WHO-approved TAS. However, surveillance of humans and mosquitoes is recommended for future programs.

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