

VECTOR SURVEILLANCE FOR LYMPHATIC FILARIASIS AFTER MASS DRUG ADMINISTRATION IN AN ENDEMIC AREA: A CASE STUDY IN BEKASI

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Abstract

Introduction: Lymphatic Filariasis (LF) re-transmission in endemic areas that have completed mass drug administration (MDA) should be a concern. Entomological data are required to support the elimination of LF. This research aims to present bionomic and evaluative evidence of *Wuchereria bancrofti* in *Culex quinquefasciatus* in Bekasi. **Methods:** Entomological surveys were carried out in Jatimulya Village, Bekasi, from October to November 2019. Female *Cx. quinquefasciatus* were caught using Human-baited Double Net traps (HDNs) both indoors and outdoors over a 12-hours (from 6 PM to 6 AM). Female mosquitos were subjected to ovary dissection to determine their longevity. In addition, the Man-Hour Density (MHD), Man-Biting Rate (MBR), Daily Survival Rate (DSR), and estimated longevity were calculated. *Wuchereria bancrofti* was detected using the Polymerase Chain Reaction (PCR) on dissected mosquitos. **Results and Discussion:** In total 673 female *Cx. quinquefasciatus* were collected. *Culex quinquefasciatus*' peak landing time was demonstrated between 12 and 3 AM. The values of Mosquito Parity Rate (MPR) and DSR are 22.88 and 0.692, respectively, implying that the estimated lifespan of dissected mosquitos ranged up to three days. The PCR analysis has revealed that none of the 48 pooled samples of *Cx. quinquefasciatus* are tested positive for *W. bancrofti*. **Conclusion:** Although this survey has found non-existent microfilaria in the LF vector *Cx. quinquefasciatus*, routine vector monitoring, and surveillance are still required to ensure the long-term viability of the LF elimination program.

INTRODUCTION

The World Health Organization (WHO) classifies Lymphatic Filariasis (LF) as a neglected tropical disease and the most common cause of physical disability. Besides social stigma, the resulting physical disability will lead to psychological pressures and reduce the economic productivity of the sufferers (1–4). LF affected 863 million people in 50 countries worldwide (in 2020). To prevent the spread of parasitic infections, preventive chemotherapy is needed (5). It is globally estimated that 25 million men suffer from lymphatic filariasis with hydrocele and more than 15 million people suffer from lymphatic filariasis with lymphoedema. These chronic disease manifestations affect at least 36 million people (5). The global prevalence has decreased, but the

cases in Southeast Asia and Africa are less likely to achieve infection prevalence thresholds suggested for local elimination (6). In Indonesia, lymphatic filariasis is significant as a major public health concern. *Brugia timori*, *B. malayi*, and *Wuchereria bancrofti* are the three species of filarial worms found in Indonesia and cause the disease. *Brugia timori* is the only species found in the eastern region of Indonesia, such as East Southeast Nusa (6–7).

In 2000, WHO launched the Global Program to Eliminate Lymphatic Filariasis (GPELF), which has two phases in its elimination strategy. The first phase is to prevent the spread of LF infection by administering Diethylcarbamazine (DEC) and albendazole (single doses) once a year for a period of 5 years (Mass Drug

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Administration/MDA). The second phase is to prevent morbidity and disability management by ensuring clinically infected people in LF endemic areas, who have access to health care facilities (8–9). Indonesia as one of the LF endemic country has launched the LF elimination program in 2005 through decree number 1582/Menkes/SK/XA/2005 concerning guidelines for LF. The Ministry of Health annually declares MDA-LF (Diethylcarbamazine/DEC and Albendazole) program through the LF Elimination Month (BELKAGA); the program is held simultaneously in October every year for five years, particularly in endemic areas with a microfilaria (Mf) rate of more than 1% (10). Until 2021, of 514 districts, in Indonesia, 236 of them are endemic areas; however, only 190 districts have Mf rates under 1% (11). The Ministry of Health of the Republic of Indonesia reports that 88 districts have completed the MDA-LF for five years in a row, and 30 districts passed the evaluation stage known as the Transmission Assessment Survey (TAS) (12).

The program is required to carry out surveillance efforts to identify the possibility of re-emergence after the MDA has stopped. WHO currently recommends post-MDA surveillance using pre-TAS (MF population prevalence) and TAS (antigen prevalence) in children aged 6-7 years old (primary schools) and born during MDA. The goal of TAS is to determine whether an area has no LF transmission so that treatment can be discontinued. The TAS design can be carried out in an area of no more than two million population per evaluation unit (EU). The survey population consists of children average aged 6-7 years in the community or grades 1-2 in elementary school. The survey design to determine TAS activities is based on the type of dominant vector species, the active enrollment rate of school children ($\geq 75\%$), the total number of children aged 6-7 years, and the number of elementary schools in the survey area. This calculation is used to determine the prevalence threshold; if the prevalence is below the threshold, the transmission is estimated to no longer continue, even without carrying out the MDA, namely “a critical cut off value”. If the positive amount of samples is equal to or below the set critical cut-off value, the government can discontinue the MDA; however, if the critical cut-off value is exceeded, treatment is repeated for two rounds of MDA (13).

Several countries have conducted TAS, but not all of them gain successful results. Several regions have at least one failed TAS, and such a condition increases resource requirements. Many factors influence the success of a TAS, including management, socioeconomic aspects, community behavior, and the

environment (presence of reservoirs and mosquito vectors). These factors have contributed to the re-transmission of LF in an area. A systematic review study has discovered that 39 countries in South America, Africa, Asia, and Latin America implemented the TAS from 2011 to 2017, and there are 936 TAS records. The study has also discovered that the risk of TAS failure in several environmental variables increases across the geographic boundaries of the evaluation unit (EU). Moreover, the study has revealed that variables, such as population density, nighttime lights, quality of living conditions, socioeconomic status, and the presence of *Brugia spp.* and *W. bancrofti* by country level are all significantly associated with failure in the evaluation of LF transmission (14).

Furthermore, it is critical to monitor the post-MDA or post-three round TAS adjustments in LF transmission to ensure that low transmission is maintained and no re-transmission occurs (15). The re-emergence of infection is a situation that must be avoided, especially in areas that have completed the MDA program or have achieved elimination. The PCR method is used to detect filaria DNA in mosquitos as an indirect indicator to describe the infection that occurs in the community (16). The vector infection rate, within certain limits and methods, is a warning of community transmission and usefully supports the success of the LF elimination program (17). For example, the detection of *W. bancrofti* DNA in America Samoa (From 1999 to 2003, Samoa underwent five rounds of MDA) mosquito species by PCR is shown positive of LF (18). These findings denote that regions, which have implemented MDA and passed the evaluation stage (TAS), are still necessarily monitored because re-emergence possibly occurs. The presence of genetic variation in vectors is also evaluated in several endemic countries, including Togo, Brazil, Egypt, Sri Lanka, India, and Bangladesh (19–24).

Two rounds of LF transmission assessment were carried out in Bekasi District in 2018 to assess the efficacy of the MDA program. Yet, the recent *Microfilaria* status in mosquitoes is unclear. Vector surveillance is critical in each post-TAS period to monitor the presence of filarial worms in nature and to prevent the occurrence of new transmissions. Understanding the bioecology of vector mosquitoes, particularly in post-MDA endemic areas, is one of the strategies to prevent the recurrence. This understanding aims to improve vector control designs and early warning systems. The goal of this study was to identify *W. bancrofti* in *Culex quinquefasciatus* as a suspected vector in Bekasi District.

METHODS

Study Area

The study was conducted during the beginning of the rainy season from 30 October to 3 November 2019 in a selected LF endemic village in Jatimulya, Tambun Subdistrict, Bekasi District, Indonesia. This district borders Jakarta and Bekasi City in the west; the two cities are a separate administration from the regency. Moreover, the district borders Bogor in the south and Karawang in the east, with coordinates of 6°21'57"S 107°10'23"E. The selected survey area in two locations consisted of 11 hamlets and 16 hamlets. These sites were selected based on the existence of LF chronic cases and spot sites (microfilaria rate > 1%). The research site was an LF-endemic area that had undergone MDA-LF for five years.

Mosquito Sampling Methods

The catches were performed using the human-baited double net trap (HDN). One adult occupied one trap and collected mosquitos for 12 hours (06 PM-06 AM). The trap consisted of a large screen tent of approximately 2 m x 1.5 m with a 1.5 m height. One tent was used for each host. The participants rested on a small screen tent of approximately 2 m (length) x 0.9 m (width) with 1.2 m height. A larger tent had six screened sides. A small tent set inside the larger tent had five screened sides. Both tents had two sides for participant entry, and the participants act as a host and were protected in a smaller tent (Fig. 1). Mosquitoes may enter the tent from ±20-30 cm gap between the two nets.

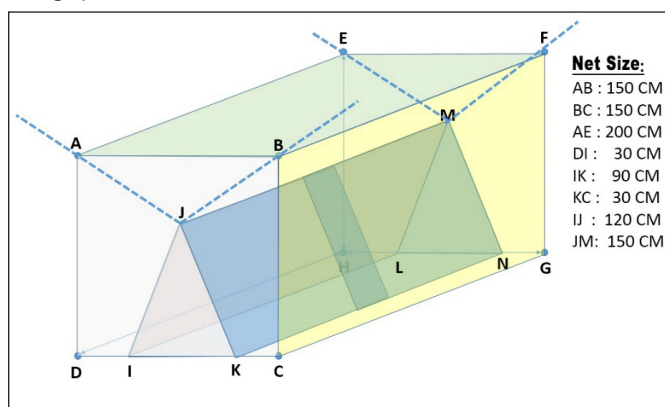


Figure 1. Human Baited Net Trap (HDNs)

Mosquito collection activities were conducted in three randomly selected houses in each cluster. The selected houses were close to the potential habitat and/or the home of the LF sufferer. Moreover, the researchers used another house to identify and prepare samples of the captured mosquitoes. This activity necessitates two mosquito collectors per house, one inside and one outside the house. Thus, there was a total of six collectors in one

cluster. Mosquito collectors were residents who were previously trained by researchers to catch mosquitos for one day and functioned as attractants (sleeping in a mosquito net). The collectors entered the inner mosquito net for 40 minutes, then came out to catch resting mosquitoes trapped in the outer mosquito net for 10 minutes. Afterward, the collectors took a break before continuing to catch mosquitoes for the next hour. The collectors caught the mosquitos resting in the mosquito net (between the inner and outer trapping nets) and placed them in a paper cup labeled with the time and location of capture. Other collectors picked up the paper cups every hour and transported them to the catching station for identification and dissection.

The mosquitoes captured were morphologically identified using regional key books entitled “Illustrated keys to the mosquitoes of Thailand IV Anopheles” (25). Meanwhile, the mosquito ovary was dissected using the WHO method, namely the “Manual on practical entomology in malaria” (26). Female *Cx. quinquefasciatus* were dissected for parity, and this process was recorded. The samples were stored for maximum of 20 mosquitoes based on location (indoor-outdoor) and dilatation (nulliparous-parous) in 1.5 mL tubes. The tubes were labeled with collection information and a unique identifier and then stored in a mosquito box. Molecular analysis was done using the PCR method. Meanwhile, the examination was conducted in Salatiga of Vector and Reservoir Control Research and Development Laboratory, the National Institute of Health Research and Development (NIHRD), the Ministry of Health of Indonesia.

DNA Extraction and PCR Analysis

Deoxyribonucleic Acid (DNA) was extracted from the head and thorax parts of mosquitoes. DNA samples were investigated using a Kit (Qiagen, Hilden, Germany). Primers NV1 (CGT GAT GGC ATC AAA GTA GGG) and NV2 (CCC TCA CTT ACC ATA AGA CAA C) were used to amplify the express sequence tags (EST), and the primers highly repeated filarial DNA sequences and homologous sequences in the genome of *Wuchereria sp* (27). The GoTaq® Green Master Mix (Promega, Madison, WI, USA) was employed in the PCR reactions. The PCR thermocycling conditions were as follows: 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and elongation at 72°C for 2 minutes followed by a final extension step at 72°C for 10 minutes. After being separated by 2.0% agarose gel electrophoresis, the amplified PCR products were visualized using the

SYBR® safe DNA gel stain (Invitrogen, Carlsbad, CA, USA). A 188-bp DNA ladder was used to calculate the size of the PCR products. To purify amplification products, Applied Biosystems ExoSAP-IT™ (Thermo Fisher Scientific, Vilnius, Lithuania) was used.

Data Analysis

Data on caught mosquitoes have been entered, and the data had been cleaned before being analyzed to calculate the man-hour density (MHD), man-biting rates (MBR), parity rate (PR), daily survival rates (DSR), and longevity of the suspected LF vector. The analyses of MBR and MHD were based on a manual on practical entomology in malaria (26). Meanwhile, measures of daily survival values (longevity) and the estimated population age of mosquitoes were based on essential malariology (28).

$$\text{(Man Hour Density/MHD)} = \frac{\text{total of mosquitoes caught}}{\text{total of collectors} \times \text{total hours time collection}}$$

$$\text{(Man Biting Rate/MBR)} = \frac{\text{total of mosquitoes caught}}{\text{total of collector} \times \text{total day time collection}}$$

$$\text{longevity (daily survival value)} = \sqrt[B]{P}$$

P = probability of mosquitoes life (daily)

A = physiological of mosquitoes age (day)

B = Parous proportion

$$\text{Estimated mosquitoes population aged} = \frac{1}{-\log_e P}$$

p = probability of mosquitoes life

log e = ln e (natural logarithm)

RESULTS

Distribution of Lymphatic Filariasis Patients

Figure 2 shows the distribution of LF chronic patients in Bekasi District. A total of 13 of 23 sub-districts in Bekasi District were reported to have chronic LF patients. A high number of LF chronic cases were identified in Tambun Sub-District, and Jatimulya is the sentinel spot site of LF. The data on chronic LF patients at the Jatimulya Health Center in Tambun District report six chronic LF patients (three of them died) and one patient with positive *Microfilaria* in January 2019. The age of LF patients ranges from 30-60 years old, and the majority of the patients are male (57.2%).

Bionomic of LF Vectors

The results show that *Culex quinquefasciatus* is dominant in this area. Moreover, only one species was captured during this survey. 673 female *Cx. quinquefasciatus* in Jatimulya village, Tambun Sub-

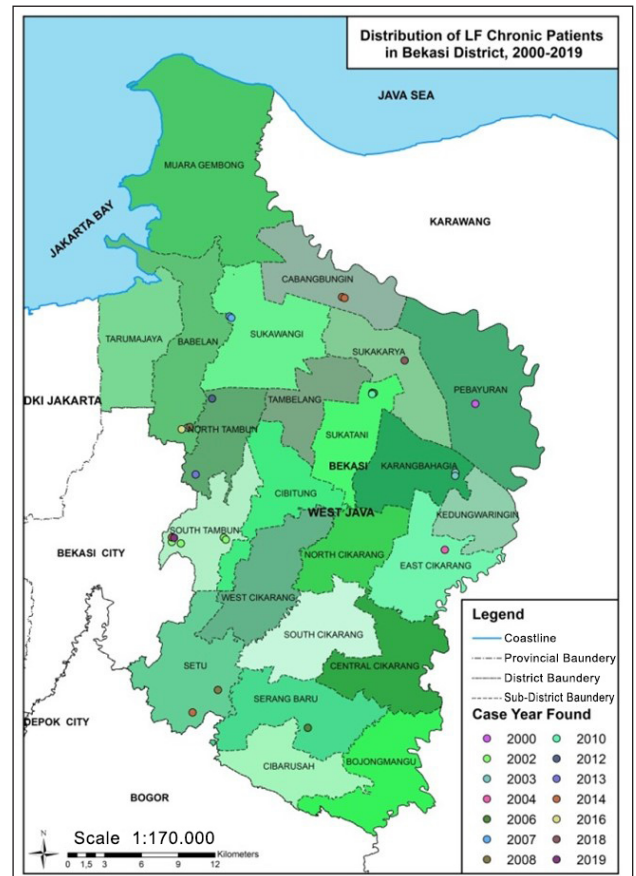


Figure 2. Distribution of LF Chronic Patients in Bekasi District

District were collected in two clusters (sub-villages 11, sub-villages 16). The results of mosquito dissection have revealed that the parity rate of *Cx. quinquefasciatus* is 22.88% and is mostly found in newly emerged. Then, 116 parous mosquitoes which were captured were dilated ≤ 3 (Table 1).

Table 1. Parity rate of *Cx. quinquefasciatus* in Jatimulya, Bekasi at October 2019

Parity	HDN Indoor	HDN Outdoor	N	%
nulliparous	179	340	519	77.12
parous			154	22.88
dilatation ≤ 3	38	78	116	
dilatation > 3	10	28	38	

*HDN = human baited double net trap

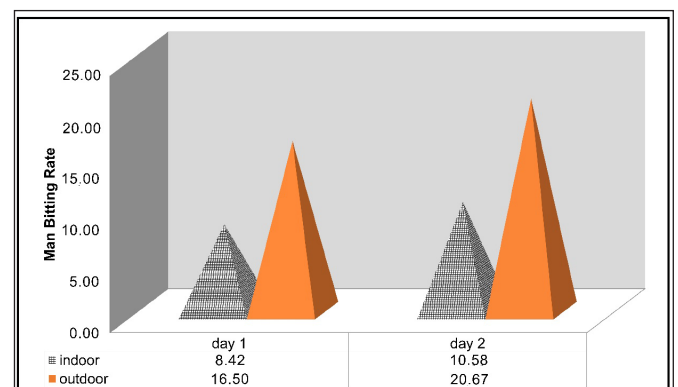


Figure 3. Man Biting Rate *Cx. quinquefasciatus* using HDN Indoor and Outdoor in Bekasi, 2019

The man-biting rates of *Cx. quinquefasciatus* are caught higher outdoors than indoors for 26.83. This score indicates that each person is bitten 19 times per night. The total MBR is 14.04 per person per night (Fig 3). Mosquito density begins to increase after 7-8 PM. The density increases at 9-10 PM and reaches a peak at 12 PM and above.

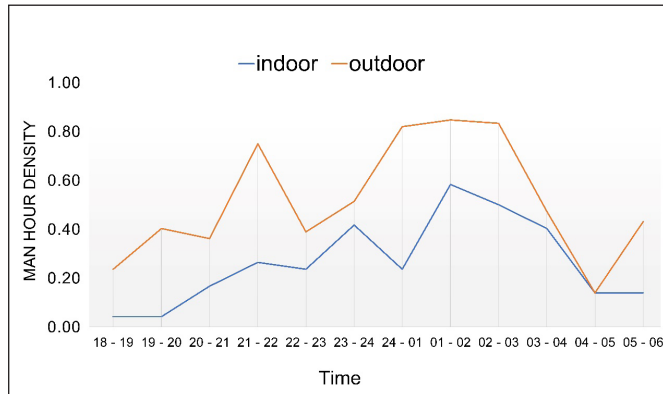


Figure 4. Man Hour Density *Cx. quinquefasciatus* using HDN Indoor and Outdoor in Bekasi, 2019

The density decreases at 4-5 AM but increases again at 5-6 AM only in outdoor traps. *Culex quinquefasciatus*'s peak landing time is between 12-2 AM. Meanwhile, the data on man-hour density when collecting the mosquitoes were 0.39 per person per hour (Fig.4). The environmental data denote that during our study period, average temperatures range between 27.8-29.5°C.

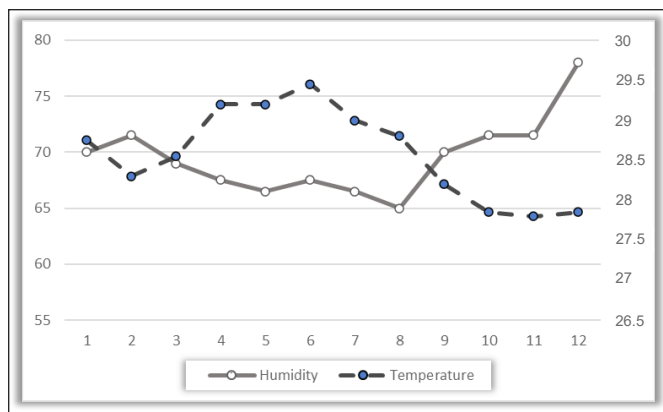


Figure 5. Average Temperature And Relative Humidity Per Hour (12 Hours) of Mosquitoes Capture in Bekasi, 2019

The temperature decreases from 11 PM, with an average relative humidity (RH) of 65-78%. The RH increases from 1 AM (Fig. 5). The parity rates and DSR of *Culex quinquefasciatus* females are 22.88 and 0.692, respectively, implying that the lifespan ranges up to three days. The estimated age population of *Cx. quinquefasciatus* in Jatimulya village recently emerges from exclusion larvae to mosquitoes (Table 2.).

Table 2. Estimated Infective Age of *Cx. quinquefasciatus* in Bekasi, October 2019

Parameter	Index
Parous	154
Parous proportion	0.2288262
Gonotrophic cycle ‡ (day)	4
Daily survival value (p)	0.6916341
(-) Ln B	-0.368698
Estimated population age (day)	2.703

‡ The research was conducted in Mexico in 2008 (50).

Detection of *Wuchereria bancrofti* by PCR

The Polymerase Chain Reaction was used to identify *W. bancrofti* in 48 pools of female *Cx. quinquefasciatus* (Fig. 6). It is examined that 48 pools do not show *W. bancrofti*. 673 samples of mosquitoes are clear from LF. It is reported that *W. bancrofti* is the main cause of LF in West Java, particularly in urban areas of Bekasi District.

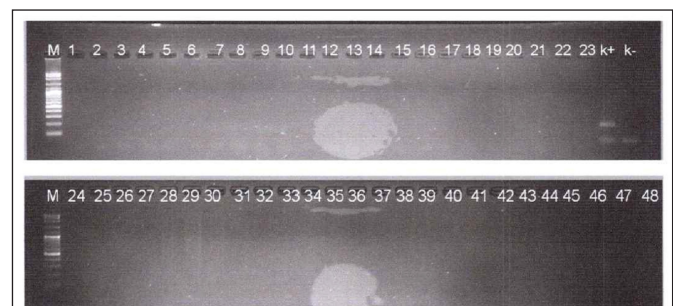


Figure 6. Identification of *W. bancrofti* in *Cx. quinquefasciatus* (48 Pooling) in Bekasi, 2019

DISCUSSION

This study describes that *Cx. quinquefasciatus* is the dominant mosquito captured in the area. Previous research has revealed that *Cx. quinquefasciatus* predominate in a number of provinces, including Banten, West Java, Lampung, East Java, North Maluku, and Maluku (29), and in many districts, including Tasikmalaya (30), Subang (31), Bogor (32), and Tangerang (33). The major LF vector in Indonesia is *Cx. Quinquefasciatus* and has been found in many parts of the country, such as Papua, Aceh, West Java, Central Java, and Jakarta (34). A high density of vectors increases the risk of disease transmission (35). *Culex quinquefasciatus* is the numerous mosquito and dominant species captured in Bekasi. Therefore, *Culex quinquefasciatus* has a chance to be a vector in the research site.

W. bancrofti is reported as the main cause of LF in West Java, particularly in urban areas of Bekasi District. In Indonesia, *W. bancrofti* is mostly found in Central Java, Sumatera, Kalimantan, Sulawesi, Nusa

Tenggara, Maluku, and Papua (7). The global endemic of *W. bancrofti* occurs in South and Southeast Asia, especially in Bangladesh, Northeast India, Lao PDR, and Myanmar (36). *W. bancrofti* in *Cx. quinquefasciatus* is also found in Jakarta and Semarang (37). Furthermore L3 of *W. bancrofti* in *Cx. quinquefasciatus* is detected in Tangerang District by using a molecular technique (38).

Cx. quinquefasciatus is confirmed as a primary vector of LF Bancroftian at Pekalongan (39–40). In addition, in Batanghari District, *Cx. quinquefasciatus* is a suspected vector for Brugian filariasis. To date, no evidence has shown that *Cx. quinquefasciatus* is confirmed as the *W. bancrofti* vector in the research site. Theoretically, there are four requirements to classify mosquitoes as a vector, including longevity, abundance, evidence of transmission, and source of transmission. This study has revealed that *Cx. quinquefasciatus* have not sufficed as a vector even though the density meets the requirement (22.8% of the parity rate). This condition indicates that mosquitoes caught in the research site have lower parity rates. In contrast, a study in Subang has revealed that the longevity of *Cx. quinquefasciatus* in densely populated residences is approximately 10-12 days. A study in Brazil has discovered 57% of parous females, indicating that the population of *Cx. quinquefasciatus* has a long lifespan. Although *W. bancrofti* is not found in the research site, the abundance of *Cx. quinquefasciatus* in mosquitoes is captured. Therefore, the community should be aware of this condition.

Other significant findings of this study denote that *Cx. quinquefasciatus* is the most dominant mosquito captured using the outdoor HDN method. This result is supported by a previous study conducted in Barito Kuala District in 2018, which discovers that *Cx. quinquefasciatus* bites more frequently outside the house with a peak landing time in the middle of the night to predawn (41). However, the result of this study is inconsistent with that of a study conducted in Pekalongan in 2016, which discovers that *Cx. quinquefasciatus* feeding habit is dominantly found indoors with the peak time landing variability (42).

This current study has found that *Cx. quinquefasciatus* are mostly outdoor-captured dominance mosquitoes. This result indicates that a potential risk of transmission occurs more frequently outside the house. Therefore, the community should be aware of their habitual behavior in urban areas because they spend their time outdoors at night (43). Furthermore, Bekasi is a densely populated City, and the majority of the sewers are open ditches. The density of *Cx. quinquefasciatus* has been linked to urbanization, suburbia, and agricultural land use. The relationship

between these factors is more closely related to the expansion of larval habitat in tropical habitats (44). A study in Qatar in 2020 has discovered that *Culex quinquefasciatus* has the highest larval density (72.4 larvae/dip), and there are nine habitat types live in urban areas, including fountains, irrigation, drinking water reservoirs, floating wastewater ponds, the system drains water ponds, sewage treated swamps, tire, metal, and plastic containers. Furthermore, salinity, dissolved oxygen, and temperature of water have a negative relationship with the density of *Cx. quinquefasciatus* while surface habitat has a positive relationship with this density (45). This indicates that immature *Culex* spp has a higher level of adaptation to various breeding sites than other mosquitoes (46). *Culex quinquefasciatus* mosquitoes are dominantly found in urban areas with lowlands, high-air temperatures, and rainfall in Hawaii in 2018. These factors have been linked to the increase in *Cx. quinquefasciatus* population. Meanwhile, Rancahlir in 2020 has revealed that *Cx. quinquefasciatus* is the dominant mosquito and has a relatively high density; although the research site is not an urban area, the air temperature is high so that a potential habitat is found (31). This finding agrees with the conditions at the site of this study. The temperature recorded during the mosquito capture tends to be high. In the early hours of capture, the temperature is 28°C and rises to 29°C. Meanwhile, the temperature begins to fall at pre-dawn, reaching 27.8°C. The number of mosquitoes in this area is affected by the high temperatures. Research conducted in the Galapagos Islands in 2018 has proven that mosquito density is strongly influenced by the interaction of climate, larval habitat, and human presence (47). Temperature and humidity also have a significant impact on several demographic aspects (48).

Vector control efforts must always be ongoing, particularly in high-risk areas, which are currently implementing MDA or post-MDA. After MDA stops, the process should be monitored to ensure that infection does not recur in the future (17). After completing MDA for five years and evaluating the intervention (TAS 1-3), some areas continue to report re-transmissions. Belitung Regency is an endemic area for *Brugia malayi* (16). It had received an LF elimination certificate in 2017, but positive microfilariasis was discovered in the same year and 2019 in two villages, namely Lassar and Suak Gual for 5.1% and 2.2%, respectively (16). Many factors influence the incidence of re-transmission. Therefore, zoonosis transmission should be monitored, especially in *Brugia* endemic areas. Despite the fact that *W. bancrofti* is not found in reservoirs other than humans, vector surveillance to assess the presence of this agent in mosquito vectors is always necessary.

The current standard procedure is conducted by taking human blood samples to monitor this number. The current standard protocol is used for PCR-based detection of parasitic DNA in insect vectors, called Molecular Xenomonitoring (MX). This protocol was employed to be an efficient and non-invasive alternative that can be indirectly detected. A study conducted in Srilanka in 2018 has discovered that transmission after elimination can still possibly occurs with the filarial DNA detected in vectors (49). Nowadays, the MX method is an accompaniment for plenty of TAS and has been implemented to ensure the interruption of transmission in several countries with various results (19–24). Several regions in Indonesia, which have received phases of elimination and evaluation of filariasis certification (TAS), consistently recommend entomology supervision. This recommendation is crucial and functions as an early warning system for LF transmission, especially in endemic areas.

This study has several limitations. First, this study neither describes the overall entomological evaluation nor represents the conditions at the research site because it was only conducted at two sites (sub-villages) in the LF endemic area. Second, this study is not conducted longitudinally so that information on the vector density and capacity could be obtained based on seasonality. However, the results of this study can be used as preliminary data for future research.

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CONCLUSION

This study has found no Mf of *Wuchereria bancrofti* in the LF vector *Culex. quinquefasciatus*. This condition indicates a low level of LF transmission in Jatimulya Village, Bekasi. However, such a condition should be a concern because *Cx. quinquefasciatus* mosquitoes are most dominantly captured outdoors. The mosquitoes potentially bring a risk of transmission outside the house. After completing MDA, the program should be monitored to ensure that the recurrence of infection will not occur in the future. Our findings suggest that routine vector surveillance for monitoring is still

required to guarantee the stability of the LF elimination program and to control the re-emergence of LF cases in a research site. Further study is encouraged to employ molecular xenomonitoring because it is an efficient and responsive method to identify parasite DNA or RNA in anthropophilic mosquitos. Moreover, this method can be used in conjunction with monitoring to ensure the interruption of LF transmission.

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