PENELITIAN | *RESEARCH*

The Distribution of *Culex* spp (Diptera: Culicidae) in Selected Endemic Lymphatic Filariasis Villages in Bandung District West Java Indonesia

Distribusi Culex *spp. di Wilayah Endemis Filariasis di Kabupaten Bandung Jawa Barat Indonesia*

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Abstrak. *Data entomologis terkait aspek bionomik dan distribusi nyamuk vektor lymphatic filariasis di Kabupaten Bandung masih sangat sedikit meskipun kabupaten ini sudah mengimplementasikan POPM Filariasis dari tahun 2009. Tujuan penelitian adalah mengindentifikasi spesies nyamuk potensial penular lymphatic filariasis dan habitatnya di wilayah endemis filariasis yaitu Kecamatan Majalaya, Kabupaten Bandung. Survei dilaksanakan selama 2 bulan yaitu September–Oktober 2013 di dua desa Kecamatan Majalaya. Kegiatannya adalah pencidukan larva (termasuk plotting habitat, salinitas, temperatur air, pH), penangkapan nyamuk dewasa menggunakan metode human landing (dalam dan luar rumah) serta resting (dinding rumah dan kandang ternak). Hasil penangkapan nyamuk memperoleh enam spesies yang berhasil diidentifikasi.* Culex quinquefasciatus *dan* Culex tritaeniorhynchus *adalah nyamuk yang dominan tertangkap dengan puncak gigitan antara jam 21.00-01.00 WIB. Terdapat lima tempat perkembangbiakan potensial yang teramati disekitar desa tersebut yang terdiri dari kolam ikan yang terbengkalai dan persawahan dengan salinitas 0‰, temperatur air 28,5-29°C, pH 6-7. Meskipun MHD dan MBR vektor filariasis yaitu* Culex quinquefasciatus *di wilayah tersebut relatif rendah, penularan masih dapat terjadi karena didukung dengan kondisi lingkungan dan keberadaan nyamuk vektor tersebut di wilayah ini.*

Kata kunci*: lymphatic filariasis, tempat perkembangbiakan,* Cx. quinquefasciatus*, kepadatan, Bandung*

Abstract. Bandung district has been implemented mass drug administration (MDA) program since 2009, but little is known about entomological data especially about bionomic aspects and distribution of lymphatic filariasis (LF) mosquito vectors. This study was aimed to identify potential LF mosquito species and its potential breeding sites in two LF endemic villages in Majalaya, Bandung district. The observational study was conducted in September-October 2013. Mosquito larvae were collected by a scoop and adult mosquitos were captured through indoor-outdoor human-landing and resting collection to identify species diversity and density. Six species filariasis mosquito vectors were identified. The primary LF vectors, *Culex quinquefasciatus* and *Cx. tritaeniorhynchus* were found as dominant species with peak landing time between 9 p.m.–1 a.m. Five potential breeding sites was identified near to villages including neglected fish-pool and paddy field with salinity 0‰, water temperature 28.5-29°C, pH 6-7. The man hour density (MHD) and man biting rate (MBR) of *Cx. quinquefasciatus* was relatively low, however, transmission may potentially occur due to their existence and the availability of favorable environmental conditions across the villages.

Keywords: lymphatic filariasis, breeding site, *Cx. quinquefasciatus*, man-biting density, Bandung

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INTRODUCTION

Lymphatic filariasis (LF) is neglected tropical diseases of global importance affecting lymphatic system and causing chronic disability and social stigma. It is estimated that 120 million people currently infected in 73 countries are at high risk of infection. Globally, there are more than 120 million people affected by LF and approximately 40 million people disfigured and debilitated by the disease.[1] It caused by filarial nematodes worm *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* and carried by wide range mosquitoes belong to different genera such as Aedes, Anopheles, Armigeres, Culex and Mansonia.[2] People who particularly lived in poor resource areas with limited health service access are known to be at a greater risk of infection.[3] Recent study has indicated that the rate of microfilaria (MF rate) was 3.1%, which means 6 million people have been infected and about 100 million people are at high risk.[4]

Indonesia has been considered as one of endemic country for LF as it has been widely distributed in almost all provinces in Indonesia. In 2014, there were 14,932 case in 418 districts and 235 districts has been considered endemic for LF.[5] A frequent number of LF cases have been reported in Indonesia. It has been found in 356 endemic area and most cases were found in poor rural and remote areas which provides favorable conditions for the existence of filarial worms *W. bancrofti, Brugia malayi, B. timori* and mosquito vector (Culex, Anopheles, Mansonia).[6]

During the period of 2002-2010, a total of 640 chronic cases has been reported in 25 districts in West Java. In 2010, there were 11 endemic areas including 7 districts (Bandung, Bogor, Karawang, Subang, Tasikmalaya, Purwakarta, Bekasi, and Kuningan) and 3 municipalities (Bogor, Depok, and Bekasi) with Microfilaria (MF) rate ranging from 1.0 to 5.25%. Since 2008, Bandung district has been declared as LF endemic area; the highest prevalence (microfilaria (MF) rate > 1%) was identified in two villages, Nanjung Village, Margaasih subdistrict (1.14%) and Padamulya village, Majalaya subdistrict (1.2%).[6] Based on the Survey, Bandung district is categorized as middle endemic area with *W. bancrofti*.[7] In order to reduce the LF prevalence, mass drug administration (MDA) has been implemented in 9 districts in West Java. Of which, 8 districts had completed the MDA program in 2013, including Bandung district.[8] The MDA program was carried out to target 2,592,053 inhabitants.

Although this MDA had completely implemented in Bandung district, evidence on the local entomological characteristics are very limited. It is essential to better understand the mosquito vector bio-ecology especially in LF endemic areas to provide evidence for strengthening vector control design and to support elimination program during the MDA and the post-MDA stage.[9] Our study was aimed to identify the species composition, dominant mosquito density and ecology of potential LF mosquito vectors in two villages where MDA had been implemented. Providing evidence on species composition, density, behaviour of population of LF mosquito vector would help local health authorities in planning vector-control strategies and to evaluating the LF elimination program.

MATERIAL AND METHOD

Study Sites

Survey was performed six times every two weeks during September-October 2013 in two villages (rural area), Padamulya village (7.044995°S-107.748914°E) and Sukamaju village (7.057022°S-107.751943°E), Majalaya subdistrict, Bandung District, West Java, Indonesia[10] (Figure 1). These two villages were selected based on demography and the presence of LF cases. Survey sites are located at approximately 695–702 m above sea level.

Ethical approval was obtained from Medical Research Ethics Committee of the National Institute of Health Research and Development, Ministry of Health of Indonesia (LB.02.01/5.2/KE.246/2013).

Mosquito Collection and Breeding Sites Observation

A standard mosquito collection was employed from 06.00 pm to 06.00 am. It consisted of 12x40 minutes indoor/outdoor human landing collection (IHL and OHL) method and 10 minutes resting mosquito collection at walls (RW) and cattlesheds (RC).[11] Six collectors were trained and performed a mosquito collection in three-selected house nearby the clinical cases patient of filariasis. All captured mosquito individuals were then identified based on the genus and species-level identification.

To investigate all potential breeding sites, we conducted environmental survey around the sites. Type of habitat was recorded and georeferenced using Garmin GPS navigator (Garmin Inc., Kansas, USA). Salinity, pH, water temperature and water level were also measured. All potential habitats were visually observed for the presence of mosquito larvae. A standard dipping method was employed to collect Aedes and Culex larvae.[12] Mosquito larvae collected using a standard mosquito dipper

(capacity 300 ml, with 13 cm diameter) and attached to a 1-meter wooden handle according to WHO procedures.[13] When mosquito larvae were present, 10 dips were taken on four defined points representative to the area. All captured mosquito larvae were then identified based on the genus-level identification.[14]

Data Analysis

Various indicators were calculated to estimate density including man hour density (MHD) and man biting rate (MBR). Mosquito density was calculated based on the following formulas.[13]

$$MHD = \frac{the\ number\ of\ mosquitoes\ caught\ from\ the\ same\ species}{number\ of\ collectors} \times \frac{60}{40}$$

$$MBR = \frac{the\ number\ of\ mosquitoes\ caught\ from\ the\ same\ species\ per\ night}{number\ of\ collectors} \times \frac{12}{8}$$

Relative abundance, frequency, and dominance of each species were also calculated. Relative abundance is amount of certain species of mosquitoes divided by the total of all types of mosquitoes caught. Frequency is the number of occurrences of certain species of mosquitoes in each hour divided by the total catching time. Dominance is the frequency multiplied by the relative abundance.

RESULT

A total of 298 mosquito individuals was collected during survey period of September to October 2013. Of which, we identified five species belong to genus Culex (n = 274) and one species belong to genus Aedes (n = 24) (Table 1). Table 1 summarized the number of mosquitoes by species and method used during the survey. We revealed the most dominant species, *Culex quinquefasciatus* and *Cx. tritaeniorhynchus,* accounting for 44.3% of the total number of mosquito, respectively. Both *Cx. quinquefasciatus* (30%, n = 40/132) and *Cx. tritaeniorhynchus* (66%, n = 87/132) were largely observed during human landing collection, especially outdoor. Both *Cx. quinquefasciatus* (33%, n = 43/132) and *Cx. tritaeniorhynchus* (12%, n = 16/132) was also found in the cattle sheds during resting method collection.

A small number of *Cx. hutchinsoni, Cx. vishnui,* and *Cx. fuscochepalus* were observed during the survey period. In general, we found two main types of mosquito habitat such as rice fields and fish-pools. Nine points of potential breeding sites were found (Figure 1). It consisted of five rice-fields and four puddles. Mosquito larvae were found in two rice-fields and two pools around study site. Water temperature ranged from 28.5-29°C, salinity was 0 and pH at 6-7. Plants found in pools vary widely (e.g., kale, velvetleaf, lotus, grass, crop rice). Predators were found in the breeding sites were fish, frogs, dragonfly larvae and water beetles (Table 3).
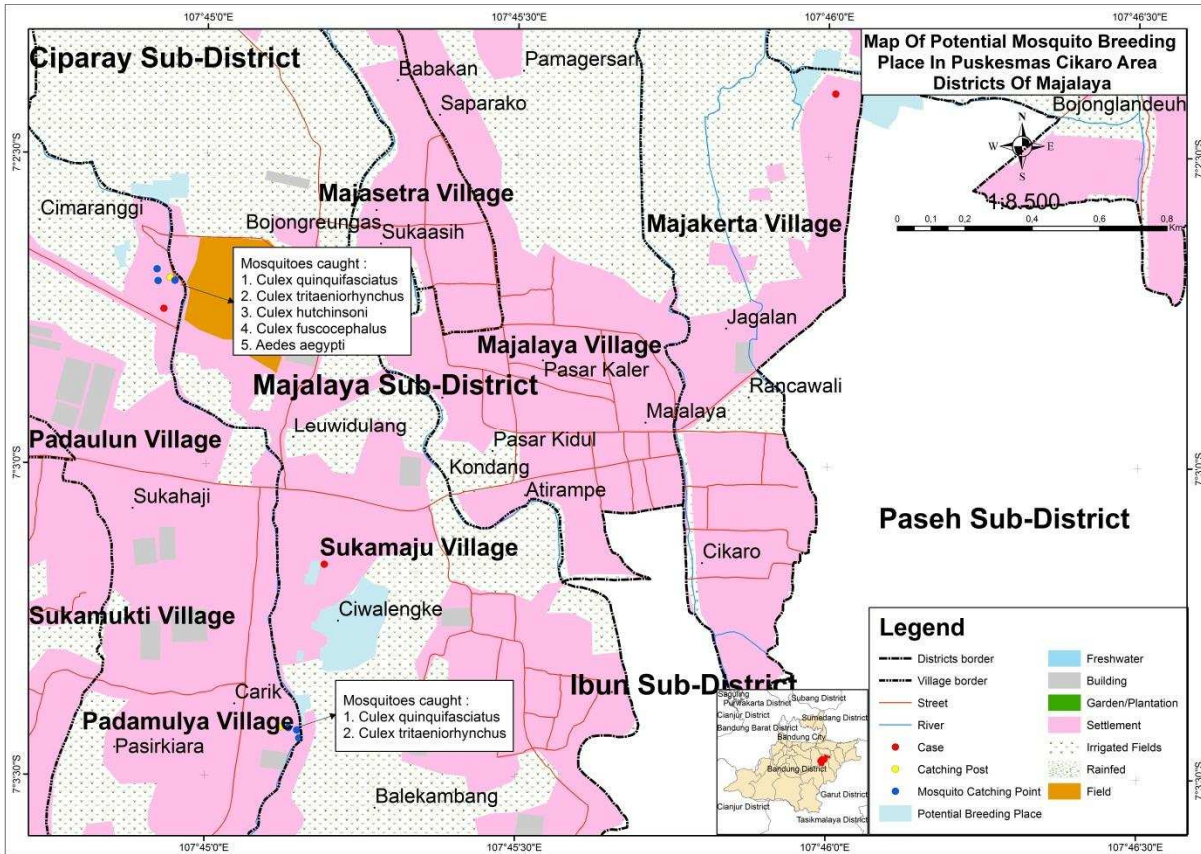
Species Composition

Table 1. Numbers of LF Mosquito Vectors Collected by Species on Each Collection Method in Padamulya and Sukamaju Village from September–October 2013

Species	Human landing		Resting		Total
	Outdoor	Indoor	Walls	Cattlesheds	
Aedes					
Ae. aegypti	0	2	22	0	24 (8.1%)
Culex					
Cx. quinquefasciatus	40	33	16	43	132 (44.3%)
Cx. tritaeniorhynchus	87	20	9	16	132 (44.3%)
Cx. hutchinsoni	3	1	1	3	8 (2.7%)
Cx. vishnui	1	0	0	0	1 (0.3%)
Cx. fuscochepalus	0	1	0	0	1 (0.3%)
	131 (44%)	57 (19.1%)	48 (16.1%)	62 (20.8%)	298

Table 2. Density, Relative Abundance, Frequency, and Dominance of LF Mosquito Vectors Collected from Padamulya and Sukamaju Village from September–October 2013

Species	Biting rate		Relative abundance	Frequency	Dominance
	Outdoor	Indoor			
Aedes					
Ae. aegypti	0.00	0.17	0.08	0.17	0.01
Culex					
Cx. quinquefasciatus	3.33	2.73	0.44	1.00	0.44
Cx. tritaeniorhynchus	7.25	1.67	0.44	0.83	0.37
Cx. hutchinsoni	0.25	0.08	0.03	0.25	0.01
Cx. vishnui	0.08	0.00	0.00	0.08	0.00
Cx. fuscochepalus	0.00	0.08	0.00	0.08	0.00

Figure 1. Distribution of mosquito breeding sites observed in Majalaya, Bandung, West Java

Table 3. Characteristics of LF Mosquito Vector Breeding Sites Observed in Majalaya, West Java

| Village	Breeding site(s)	Coordinate	Presence of larvae	Biotic factor	Abiotic factor		
					Temp (℃)	Salinity (per mil)	pH
Padamulya	Rice Field 1	S 7°2'39.7"; E 107°44'51.4"	+	*Oryza sativa*	28.50	0.00	6.0
	Rice Field 2	S 7°2'33.4"; E 107°44'55.5"	-	*Oryza sativa*	28.50	0.00	6.0
	Rice Field 3	S 7°3'23"; E 107°45'9.1"	+	*Oryza sativa*	29.00	0.00	7.0
	Pool 1	S 7°2'37.9"; E 107°44'53.2"	+	*Ipomoea aquatic*	28.50	0.00	6.0
	Pool 2	S 7°2'37"; E 107°44'52.6"	+	Grace	28.50	0.00	6.0
	Pool 3	S 7°3'24.8"; E 107°45'52.6"	-	*Limnocharisflava*	29.00	0.00	7.0
Sukamaju	Rice Field 1	S 7°3'10.5"; E 107°45'10"	-	*Oryza sativa*	28.60	0.00	6.0
	Rice Field 2	S 7°3'15.7"; E 107°45'13.9"	-	*Oryza sativa*	28.60	0.00	6.0
	Pool 1	S 7°3'18.5"; E 107°45'8.8"	-	Nymphaea	28.60	0.00	6.0
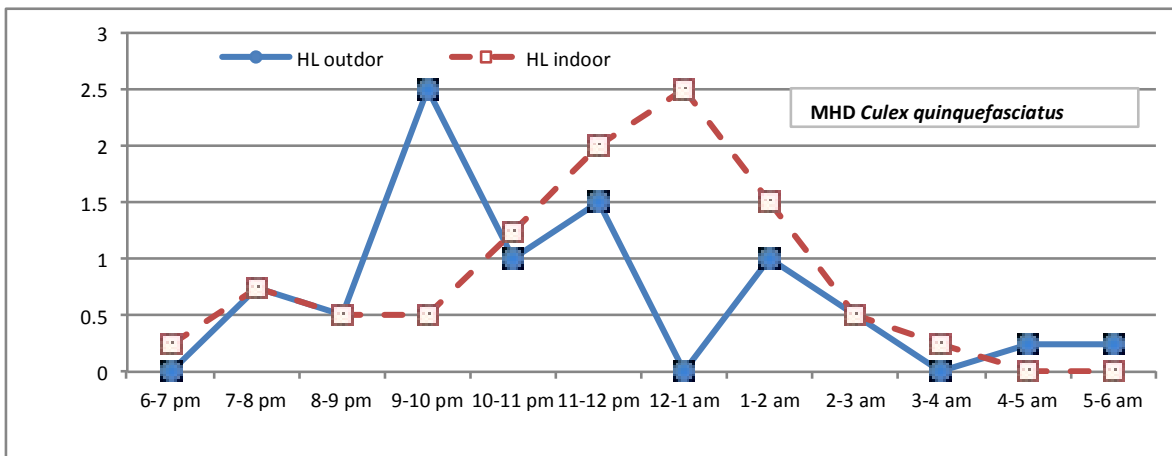
Figure 2. Man hour density (MHD*)* of *Cx. quinquefasciatus*
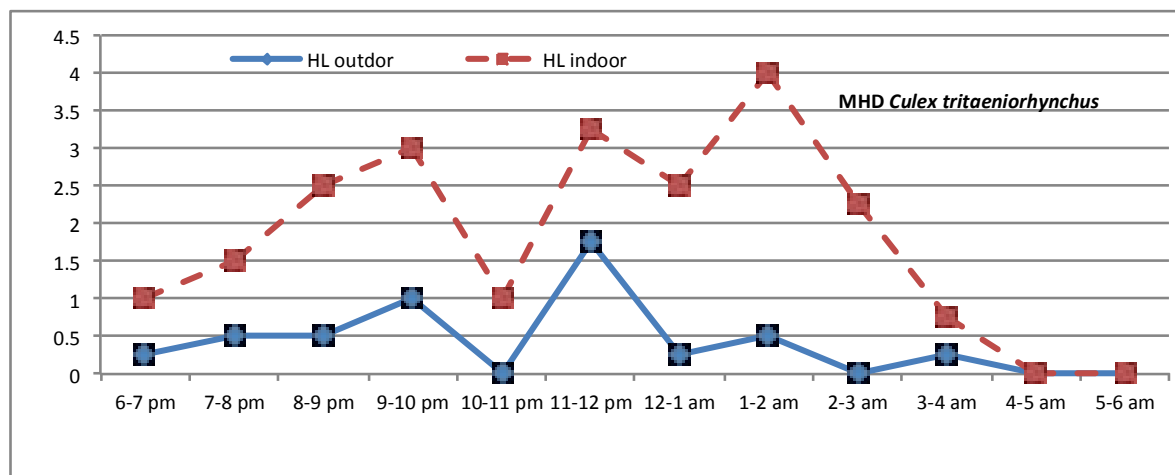
Figure 3. Man-hour density (MHD*) of *Cx. tritaeniorhynchus*

Density and Landing Pattern of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*

The mosquito density was estimated using MBR and MHD index. The man biting rate (MBR) of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* during outdoor human-landing collection was relatively higher than indoor collection, ranging from 3.33-7.25 mosquito/person (Table 2). The relative abundance index, frequency, and dominance index was high for *Cx. quinquefasciatus* (0.44; 1; 0.44, respectively).

The man hour density (MHD) of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* showed a fluctuative and distinct trend for each species. The density of *Cx. quinquefasciatus* reached a peak during 9–11 p.m. (outdoor) and 12 p.m.–1 a.m. (indoor). Whereas, *Cx. tritaeniorhynchus* was peaked at 11–12 p.m. (outdoor) and 1–2 a.m. (indoor) (Figure 2).

DISCUSSION

Our study revealed abundant LF vector mosquito *Cx. quinquefasciatus* in the villages, it was show from dominance index (0.44), this is due to explain many breeding sites was found in the area.

Culex quinquefasciatus was the dominant mosquito with highest relative abundance agree with the findings reported in other part of Indonesia such as Pekalongan[15,16], Mojokerto[17], Serang[18]. *Culex quinquefasciatus* mosquito has the highest density compared to other species found in this study and it appears to be potential LF vector in the area. Our findings indicate both *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* found in the study sites tended to be anthropophilic; suggesting potential risk of LF transmission. In addition, our study also found abundant species of *Cx. tritaeniorhynchus.*

Although it is not considered as LF vectors, the presence of *Cx. tritaeniorhynchus* in the areas have shed the light on the potential transmission of other disease such as *Japanese encephalitis* (JE).[19,20,21,22] Although MHD and MBR relatively low but the potential for transmission should be awared because population mobility in this region relatively high.

In Indonesia, *Cx. quinquefasciatus* is the major LF vector and has been documented in many parts in Indonesia including Aceh, West Java, Jakarta, Central Java, and Papua.[6] The study in Pekalongan was successfully identified microfilaria *W. bancrofti* in *Cx. quinquefasciatus* mosquito[23], while *B. malayi* has been identified in *Cx. quinquefasciatus* mosquito in the study in Batanghari district.[24]

The presence of the major LF mosquito vector *Cx. quinquefasciatus* in our study site suggests the needs to improve vector control measures in the area, as this is an endemic area. Although the mass drug administration (MDA) has been carried out, routine surveillance should be employed to monitor and control LF transmission in the areas.

Our study also indicates that *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* mosquitoes showing different temporal pattern in density during the observation. This may influence by a number of environmental factors (e.g., temperature, humidity).[25] Understanding the temporal behaviour of vectors could help to identify appropriate prevention strategies to reduce the likelihood of contact between human and vectors. This findings also show that the mosquito is a cosmopolitan species and closely associated with human dwellings.[25,26] Mosquito density peaked at night and declined shortly before sunrise. This pattern may possibly link with the dynamic changes on temperature and

humidity.[27] However, we recorded no significant changes in temperature both indoor (mean temperature = 25.12℃; RH= 68-80% with an average of 73.86%) and outdoor (mean temperature = 25.35℃; RH = 66-80% with an average of 75.2%) during the study. Studies have shown that *Cx. quinquefasciatus* shows a preference for avian blood but will feed readily on mammals, including humans.[28,29]

Mosquito biology and the environment can play important roles in mosquito vector competence, study in India shown that the probability of infected mosquitoes surviving to have complete development of filarial larvae (1 days) was 0.17. The expectation of infective life was 1.416 days for man biting *Cx. quinquefasciatus* and the adult survival rate was 87.6%. It has been estimated that a total of 22,569 mosquito bites were infective (0.8%). Monthly transmission index of *W. bancrofti* filarial showed two periods of transmission.[30]

We identified potential breeding sites around the study site, including rice-fields and puddles, which is favorable habitat for *Cx. quinquefasciatus* to maintain their population in the area.[31,32] The availability of such suitable habitats might have played significant role in resulting high abundance of LF vectors observed in this study. To maintain the LF transmission risk at low level, it is important to ensure that factors associated with vector population, such as breeding sites, monitored routinely.

The limitations of our study deserve further consideration. Short-time period of study has restricted further analysis on seasonal distribution of LF vectors and its impact on changes in density and transmission dynamics. Given that time-constraint, our study might have not represented the actual mosquito species diversity. In addition, we did not attempt to identify the existence of microfilariae in *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* due to technical and sample size constraints. However, this study given recent entomological status on the potential of LF mosquito vectors after the MDA implementation in Bandung district, which may beneficial for local health authorities.

CONCLUSION

The study has shown that *Cx. quinquefasciatus* was the dominant LF vectors mosquito identified in Majalaya subdistrict. Despite its low density, the presence of LF vectors and its breeding sites may pose a potential LF transmission risk in the villages. We suggest that routine surveillance should be carried out to maintain level of transmission to succeed LF elimination program in Bandung district.

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