

Diversity, domination and behavior of mosquitoes in filariasis endemic area of Bogor District, West Java, Indonesia

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Abstract. Nirwan M, Hadi UK, Soviana S, Satrija F, Setiyaningsih S. 2022. Diversity, domination and behavior of mosquitoes in filariasis endemic area of Bogor District, West Java, Indonesia. *Biodiversitas* 23: 2093-2100. A study on the diversity, dominance and behavior of mosquitoes was done in the two filariasis endemic areas in Bogor District, West Java, Indonesia. The objectives of this study were to determine the diversity, dominance and behavior of mosquitoes and their importance as vectors of filariasis in two endemic areas in Bogor District, namely Tamansari Village (rural) and Cimanggis Village (urban). This research was carried out from September 2019 to February 2020. Mosquitoes were collected from 6.00 pm to 6.00 am, twice a month in each village, by using the bare leg collection technique. The collected mosquitoes were identified and analyzed. Filaria detection was carried out by dissecting technique and polymerase chain reaction (PCR). The result indicated eight (8) species of mosquitoes in the rural area (Tamansari village), i.e., *Culex quinquefasciatus*, *Aedes aegypti*, *Cx. vishnui*, *Cx. tritaeniorhynchus*, *Armigeres kesseli*, *Ar. subalbatus*, *Ae. albopictus*, *Mansonia annulata*. On the other hand, there were six (6) species found in the urban area (Cimanggis village), i.e., *Cx. quinquefasciatus*, *Ae. aegypti*, *Ar. kesseli*, *Ar. subalbatus*, *Cx. vishnui*, *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* were found to be the most dominant in Tamansari village (90.46) and also in Cimanggis Village (95.67). Based on the analysis showed that the mosquito diversity index was low in both the Tamansari village (H' : 0.444) and the Cimanggis village (H' : 0.238). In general, mosquito-biting behavior prefers to suck blood indoors (anthropophilic) with a peak density in the range of 23.00-04.00. The results of filarial detection using dissecting technique and PCR methods against mosquitoes caught in Tamansari Village and Cimanggis Village did not find any L3 larvae and microfilariae in the examined mosquitoes. *Culex quinquefasciatus* was found to be very potential as the main vector for the spread of filariasis both in rural and urban areas of endemic filariasis of Bogor District, Indonesia.

Keywords: Behavior, diversity, dominance, filariasis endemic, mosquitoes

INTRODUCTION

In Indonesia, lymphatic filariasis is a disease caused by filarial worms of the species *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* transmitted by several species of mosquitoes of the genus *Mansonia*, *Culex*, *Anopheles*, *Armigeres*, and *Aedes* (Kementerian Kesehatan 2014). This disease is still a global health problem and the second leading cause of disability in the world (WHO Regional Office for South-East Asia 2010) and was one of the diseases that WHO must eliminate by 2020, which was later revised to 2030 (Chu et al. 2010; Hooper et al. 2014; Chandrasena et al. 2018; Prada et al. 2021). This disease is one of the neglected diseases that can cause physical disability, stigma, poor psychosocial and loss of work and earnings (Abdulmalik et al. 2018; van 't Noordende et al. 2020; Ali et al. 2021; Istianah et al. 2021). Lymphatic is characterized by the discovery of adult filaria worms living in the lymphatic system (Chandy et al. 2011). These worms can cause acute clinical manifestations in the form of recurrent fever, inflammation of the lymphatic system. The main clinical manifestations are acute adenomyitis and

chronic lymphedema, which causes elephantiasis in both men and women and can form hydroceles in men (at least in *Wuchereria bancrofti*) (Babu and Nutman 2014).

Filariasis was widespread throughout Indonesia and there were some areas with high endemicity. The increase in cases of filariasis occurred from 2002 to 2014. In 2015, there was a decrease in cases from 14,932 patients to 13,032 patients. The provinces with the most clinical cases in 2015 were East Nusa Tenggara (2864 patients), Aceh (2372 patients), and West Papua (1244 patients), while the provinces with the lowest cases were North Kalimantan (11 patients), NTB (14 patients) and Bali (18 patients). In 2016, there were 29 provinces and 239 regencies or /cities reported as endemic areas, an estimate of people at risk of filariasis infection as many as 102,279,739 patients (Pusdatin Kemenkes RI 2016).

In Indonesia, there are 23 species of mosquitoes from 5 genera, namely: *Mansonia*, *Anopheles*, *Culex*, *Aedes* and *Armigeres*, which are vectors of filariasis (Kementerian Kesehatan 2014). *Brugia malayi* was transmitted by *An. barbirostris*, *Mansonia uniformis* and *M. bonnea*. *Brugia timori* was found in limited areas of Eastern Indonesia such

as Timor, Flores, Rote, the Alor islands and the small islands of East Nusa Tenggara and Southeast Maluku. The vector (transmitter) of *B. timori* was *An. barbirostris*. However, urban *Wuchereria bancrofti* was transmitted nocturnally by *Culex quinquefasciatus*, which lives in domestic wastewater and has been found in urban areas such as Bekasi, Tangerang, Pekalongan and Lebak in Java. In contrast, rural *W. bancrofti* was found outside Java, particularly in Papua and East Nusa Tenggara, and is transmitted nocturnally by *Culex* sp. and *Anopheles* sp. (*An. gambiae*, *An. funestus*, *An. scapularis*) (Lee and Ryu 2019).

Cimanggis village Bojong Gede subdistrict and Tamansari village Rumpin subdistrict are two filariasis endemic areas in Bogor District. Cimanggis village has an area of 42.8 ha with the northern boundary of Sukmajaya village, south of Kayu Manis village, east of Kedung Waringin village, West of Kemang village. Cimanggis village has industrial technology and services, with the main livelihoods are civil servants and private employees. The number of Cimanggis villagers is 17,968 people, with a number of family heads (KK) of 4693. Tamansari village has an area of 983.5 ha with the northern boundary of Sukamulya village, on the south bordering Sukasari village, east bordering Gunung Sindur sub-district and west bordering Kertajaya village. Demographically Tamansari village has a plantation with an area of 450 ha. The main livelihoods are private and self-employed employees. The population of 11,674 people with a number of households of 2955.

According to Dhimal et al. (2014), entomological surveys are very important in controlling vector populations and vector infectious diseases in society. The entomological survey will show vector distribution and disease transmission capacity. Knowledge of the bionomics of the species involved in disease transmission is very important in vector control programs (Amin et al. 2013). As endemic areas in Bogor Regency, Cimanggis Village and Tamansari Village, it is necessary to conduct an entomological survey to determine the bio-ecology of mosquitoes, including the diversity and dominance of mosquitoes, so that potential vectors of filariasis and mosquito-biting behavior and parity conditions can be identified.

MATERIALS AND METHODS

Study area

The research was conducted in two villages, namely Cimanggis Village, Bojong Gede Subdistrict, and Tamansari Village, Rumpin Subdistrict, Bogor District, West Java, Indonesia. The choice of the two regions due to these areas are the area with the highest cases of filariasis and located in two different typological areas.

Procedures

The collection of adult mosquitoes was carried out 12 times for 6 months (September 2019 to February 2020) for each village. Mosquito sampling was conducted according

to WHO standards (2003) with the Human Landing Collection (HLC) and Resting Collection method for 12 hours of observation (06.00 pm-06.00 am). Mosquito catching was carried out by collectors as well as bait inside and outside the house. The study used 6 collectors, each 3 inside the house and 3 outside the house. The house that was sampled began in the house where there were filariasis sufferers and near the mosquito habitats. The collection technique was carried out every hour for 45 minutes for mosquitoes to be caught with people's bait and sucked using an aspirator, then 10 minutes for catching those who were resting inside and outside the house. The collected mosquitoes were put into paper cups and then be identified.

Parousity examination was carried out by dissecting mosquito ovaries. The identified mosquitoes were placed in a petri dish, the wings and legs were separated from the body, then the mosquito body was placed on a glass object and dropped with physiological NaCl. After that, surgery was performed using a surgical needle. Surgery was performed microscopically using a stereomicroscope. The surgical needle in the left-hand presses on the chest, and the surgical needle in the right-hand presses on the VIII segment and then slowly shift it to the right until the contents of the abdomen and ovaries are pulled outward. The ovaries were placed on a glass object that had just been given distilled water to view the tracheolar (*tracheolus skein*) using a light microscope with a magnification of 40x10 (WHO 1975).

The mosquito dissecting technique to detect filarial larvae refers to WHO (2013). Fresh mosquitoes were used for the dissection were fresh mosquitoes, and if they were dead, dissecting must be carried out immediately within 6 hours or the next day if stored at 4°C. The mosquitoes that were examined for their filarial larvae were mosquitoes with *parous* status. Mosquitoes were placed on a petri dish, and their wings and legs were removed using two pairs of needles or forceps and then observed under a stereoscopic surgical microscope at low power magnification. Mosquitoes were placed on a microscope slide, and their body parts were separated with a surgical needle into the head, thorax and abdomen. Furthermore, each body part was dripped with physiological saline solution on the same slide. The three separated body parts were examined for larval or microfilariae stage worms. The mouthparts should be separated with a fine needle to allow the L3 larvae to escape and be seen moving in the saline drop. A compound microscope with a magnification of 40x was used as it is better for finding all worms. The location and number of worms in each body part were recorded. Stages L1 and L2 are frequently seen in the thoracic flight muscles, whereas L3 is commonly found in the head and neck region or out of the proboscis, as well as in the thoracic *haemocoel* (mosquito body cavity). In freshly sucked blood, the midgut can be removed from the body and the blood cells lysed in distilled water. The microfilariae can then be counted in the blood (under a 40x compound microscope).

Mosquito examination by PCR adopts the method that has been carried out by Santoso et al. (2015) method using "*chelex 100*".

Data analysis

Diversity and relative abundance

The data were descriptively analyzed and calculated relative abundance figures, captured frequencies, and dominance figures of each species. Relative abundance is a comparison between the number of mosquitoes of a species with the number of all mosquitoes of different species collected, expressed in percent.

$$\text{Relative abundance} = \frac{\text{the number of mosquitoes of a species}}{\text{total species mosquitoes collected}} \times 100\%$$

The frequency of caught mosquitoes was calculated based on the comparison between the number of collections obtained by a certain mosquito and the total collection of mosquitoes. The dominant figure of mosquito species is calculated from the result of the multiplication of relative abundance with the frequency of mosquitoes collected by certain species.

$$\text{Frequency} = \frac{\text{the number of collections obtained by certain mosquito}}{\text{the total collection of mosquito}}$$

The dominant figure = Relative abundance x Frequency

The species diversity index (H) is calculated using a formula according to Shannon-Wiener (Magurran 2004).

$$H = - \sum pi \ln pi$$

Where, H: The species diversity index; pi : The proportion of species is a comparison between the number of mosquitoes of a species and the number of all mosquitoes of different species collected.

Mosquito density

The density of adult mosquitoes was analyzed descriptively by using the mosquito index, namely Man Hours Density (MHD). MHD is the number of mosquitoes that bite people per hour at a specific time of the day. MHD is determined by the number of mosquitoes that land on a feeder's limb and are successfully captured, divided by the number of catchers multiplied by the capture time in hours (Istianah et al. 2021). MHD is based on the equation as follows:

$$\text{MHD} = \frac{\sum \text{species mosquitoes collected}}{\sum \text{collector} \times \text{time collection (hours)} \times \text{time collection (minutes)}}$$

RESULTS AND DISCUSSION

The mosquitoes collected during the study were 4340 individuals in Tamansari village and 2747 individuals in Cimanggis village. Tables 1 and 2 show the diversity, relative abundance, frequency, dominance, and mosquito diversity index in Tamansari village and Cimanggis village of Bogor Regency. Based on Table 1, it can be seen that there were 8 types of mosquito species found during the study in Tamansari village. The highest dominance was in the species *Cx. quinquefasciatus* (90.46) and the lowest dominance in the species *Mansonia annulata* (0.01), the mosquito species diversity index was 0.444. Table 2 shows that there were 6 species of mosquitoes in Cimanggis village, with the highest dominance in *Cx. quinquefasciatus* species of 95.67 and the lowest in *Cx. tritaeniorhynchus* species of 0.02. The mosquito diversity index was 0.238.

Table 1. Diversity, relative abundance, frequency, dominance and mosquito diversity index caught in Tamansari Village, Rumpin Subdistrict Bogor District during September 2019 to February 2020

Species	n	Relative abundance (%)	Frequency	Dominance	H'
<i>Cx. quinquefasciatus</i>	3926	90.46	1.00	90.46	0.444
<i>Cx. vishnui</i>	80	1.84	0.50	0.92	
<i>Ae. aegypti</i>	229	5.28	1.00	5.28	
<i>Ma .annulata</i>	3	0.07	0.17	0.01	
<i>Cx. tritaeniorhynchus</i>	31	0.71	0.58	0.42	
<i>Ar. kesseli</i>	25	0.58	0.50	0.29	
<i>Ar. subalbatus</i>	32	0.74	0.42	0.31	
<i>Ae. albopictus</i>	14	0.32	0.25	0.08	
Total	4340	100			

Table 2. Diversity, relative abundance, frequency, dominance and mosquito diversity index caught in Cimanggis village, Bojong Gede Subdistrict Bogor District during September 2019 to February 2020

Species	n	Relative abundance (%)	Frequency	Dominance	H'
<i>Cx. quinquefasciatus</i>	2628	95.67	1.00	95.67	0.238
<i>Cx. vishnui</i>	9	0.33	0.33	0.11	
<i>Ae. aegypti</i>	27	0.98	0.92	0.90	
<i>Cx. tritaeniorhynchus</i>	4	0.15	0.17	0.02	
<i>Ar. kesseli</i>	44	1.60	0.33	0.53	
<i>Ar. subalbatus</i>	35	1.27	0.58	0.74	
Total	2747	100			

Tables 3 and 4 show the diversity, relatives, abundance, frequency and dominance of mosquitoes collected indoors and outdoors biting, indoor and outdoor resting in Tamansari village and Cimanggis village in Bogor district. Table 3 showed that *Cx. quinquefasciatus* mosquitoes bite outdoor more dominantly (91.51) than indoors (90.84), while at rest, *Cx. quinquefasciatus* mosquitoes were more dominant indoors (89.57) than outdoor resting (87.46), while for *Ae. aegypti* mosquitoes with the second most relative abundance (5.28%) showed more dominant indoor biting (5.95) than outdoor biting (3.53) while at rest *Ae. aegypti* mosquitoes were more dominant indoors (5.63) than outdoor resting (4.77). Table 4 shows the dominance of *Cx. quinquefasciatus* mosquitoes indoors biting (98.17) was greater than outdoors biting (94.33) while resting was also more dominant indoor (94.99) than outdoors resting (92.21).

The peak density of *Cx. quinquefasciatus* sucking mosquitoes inside the house occurred at 23.00-24.00, while outdoors, it occurred at 2.00 to 3.00. The same thing was also found in Cimanggis village in species *Cx. quinquefasciatus* mosquitoes with a higher blood density (units per hour) indoors than outdoors. The peak density of *Cx. quinquefasciatus* mosquitoes sucking blood in the room occurred at 22.00-23.00 while the peak of *Cx. quinquefasciatus* sucking blood outdoors was found at 23.00-24.00. (Figure 1d).

The bloodsucking activity of *Ae. aegypti* indoor and outdoor showed a declining trend. The peak of sucking mosquito blood indoors was at 19.00-20.00, while outdoor was at 20.00-21.00 (figure 1b). Bloodsucking activity *Cx. vishnui* tends to fluctuate. Peak sucking the blood of *Cx. vishnui* outdoor at 03.00-04.00 while indoors at 18.00-19.00 (Figure 1c).

The results of mosquito ovary identification, PCR test, and microscopic examination of L3 larvae in Tamansari Village and Cimanggis Village for 6 months of mosquito collection were presented in Tables 5 and 6. The highest parity rate was found in *Ar. kesseli* and *Ae. albopictus* (100%), while the lowest was in *Ma. Annulata* (33%). The highest parity rate in Cimanggis Village was found in *Cx. vishnui*, *Ae. aegypti* and *Cx. tritaeniorhynchus* (100%) and the lowest in *Ar. subalbatus* (80%). Tables 5 and 6 explain that the PCR text did not find microfilariae in the mosquito's body, and also, microscopic examination did not find any L3 larvae in the mosquito's body.

Discussion

Research conducted in two filariasis endemic areas with different typologies in Bogor District resulted in 8 mosquito species in Tamansari Village and 6 mosquito species in Cimanggis Village. Total mosquitoes caught in Tamansari Village were 4340 individuals, and in Cimanggis Village, 2747 individuals. The most dominant mosquito found in the two areas was *Cx. quinquefasciatus*. *Culex quinquefasciatus* in Tamansari Village obtained a dominance value of 90.46, while in Cimanggis Village

obtained a dominance value of 95.67. The high dominance of *Cx. quinquefasciatus* was caused because this mosquito had a high population density, and also the condition of the habitat supports the development of this mosquito species. *Culex quinquefasciatus* was the main filariasis vector in Asia, Central America, South America, East Africa, especially in tropical countries (Tennyson et al. 2012; Simonsen and Mwakitalu 2013). In Brazil, this mosquito was found as a vector of filariasis and several arboviruses in several large cities. In Youunde City, Cameroon, this mosquito was the most abundant and a major problem burden. *Culex quinquefasciatus* mosquitoes were intelligent vectors because they have many abilities to survive and thrive in both urban and rural areas. According to Bhattacharya and Basu (2016), *Cx. quinquefasciatus* creates ecological bridges between urban, suburban, and rural areas due to their presence and adaptability in many ecological niches. *Culex quinquefasciatus* can be expressed as a potential vector in these two endemic areas (Tamansari Village and Cimanggis Village) because of their highest abundance and dominance. Several studies report that vector mosquitoes in endemic areas are mosquitoes with the highest abundance and dominance (Sukatendra and Shidqon 2016).

The results also showed that the diversity of mosquitoes caught in Tamansari and Cimanggis villages was generally low, based on the Shannon diversity index that showed in Tamansari village ($0.444 < 1$) and Cimanggis village ($0.238 < 1$). This low diversity was due to the dominance of *Cx. quinquefasciatus* mosquitoes in the area due to the support of suitable habitats. The variety of insects was great in an ecosystem, indicating that the ecosystem environment was balanced or stable. Such a high insect range will enable the ordinary walking off the food net process and vice versa; that is, low insect variety in the ecosystem suggests an unbalanced and unstable ecosystem (Richard et al. 2016; Landi et al. 2018).

The analysis results showed that mosquito-biting activity in Tamansari Village was more dominant outdoors than indoors. This phenomenon occurs because, naturally, adult mosquitoes prefer to live outdoors. Environmental conditions also affect the physical, biological, and socio-cultural environment in Tamansari Village, supporting the behavior of mosquitoes that like to bite outdoors compared to indoors. A different situation occurred in Cimanggis Village; biting mosquitoes indoors was more dominant than outdoors. This situation occurs because the habits of the people in Cimanggis Village at night were more active indoors than outdoors, so the *Cx. quinquefasciatus* tries to enter the house and bites humans to get blood to ripen their eggs. Mosquitoes caught resting in both Tamansari and Cimanggis villages showed that mosquitoes prefer to rest indoors than outside. *Culex quinquefasciatus* influenced this situation, known as a house mosquito that likes to rest in the house. In addition, the mosquito *Ae. aegypti* also has a behavior that likes to rest in the house, especially in dark places.

Table 3. Diversity, relatives abundance, frequency, and dominance of mosquitoes collected indoor and outdoor biting, indoor and out door resting in Tamansari village, Rumpin sub-district Bogor District during September 2019 to February 2020

Species	Indoor biting				Indoor resting				Outdoor biting				Outdoor Resting			
	N	RA	Freq	Dom	N	RA	Freq	Dom	N	RA	Freq	Dom	n	RA	Freq	Dom
<i>Cx. quinquefasciatus</i>	1190	90.84	1	90.84	610	89.57	1	89.57	1617	91.51	1	91.51	509	87.46	1	87.46
<i>Cx. vishnui</i>	21	1.60	0.42	0.67	7	1.028	0.17	0.17	34	1.924	0.42	0.80	18	3.093	0.33	1.03
<i>Ae. aegypti</i>	78	5.95	1	5.95	46	6.755	0.83	5.63	68	3.848	0.92	3.53	37	6.357	0.75	4.77
<i>Ma. annulata</i>	1	0.08	0.08	0.01	0	0	0.00	0.00	2	0.001	0.08	0.00	0	0	0.00	0.00
<i>Cx. tritaeniorhynchus</i>	1	0.08	0.08	0.01	7	0.011	0.42	0.00	20	0.011	0.42	0.00	3	0.005	0.25	0.00
<i>Ar. kesseli</i>	5	0.38	0.33	0.13	6	0.009	0.33	0.00	11	0.006	0.25	0.00	3	0.005	0.17	0.00
<i>Ar. subalbatus</i>	8	0.69	0.25	0.17	3	0.004	0.25	0.00	11	0.006	0.33	0.00	9	0.015	0.25	0.00
<i>Ae. albopictus</i>	5	0.38	0.08	0.03	2	0.003	0.17	0.00	4	0.002	0.08	0.00	3	0.005	0.17	0.00
Total	1310				681				1767				582			

Note: n: number of mosquitoes; RA: Relative abundance; Freq: Frequency; Dom: Dominance

Table 4. Diversity, relatives abundance, frequency, and dominance of mosquitoes collected indoor and outdoor biting, indoor and out door resting in Cimanggis village, Bojong Gede sub-district Bogor District during September 2019 to February 2020

Species	Indoor biting				Indoor resting				Outdoor biting				Outdoor Resting			
	n	RA	Freq	Dom	N	RA	Freq	Dom	N	RA	Freq	Dom	n	RA	Freq	Dom
<i>Cx. quinquefasciatus</i>	1074	98.17	1	98.17	455	94.99	1	94.99	732	94.33	1	94.33	367	92.21	1	92.21
<i>Cx. vishnui</i>	2	0.18	0.17	0.03	1	0.21	0.17	0.03	4	0.52	0.25	0.13	2	0.51	0.17	0.08
<i>Ae. aegypti</i>	2	0.18	0.17	0.03	12	2.51	0.58	1.46	7	0.90	0.42	0.38	6	1.51	0.42	0.63
<i>Cx. tritaeniorhynchus</i>	0	0	0.00	0.00	0	0	0.00	0.00	2	0.26	0.17	0.04	2	0.50	0.08	0.04
<i>Ar. kesseli</i>	9	0.82	0.25	0.21	6	1.26	0.25	0.31	18	2.32	0.25	0.58	11	2.76	0.17	0.46
<i>Ar. subalbatus</i>	7	0.64	0.17	0.11	4	0.84	0.25	0.21	13	1.68	0.25	0.42	10	2.51	0.25	0.63
Total	1094				479				776				396			

Note: n: number of mosquitoes; RA: Relative abundance; Freq: Frequency; Dom: Dominance

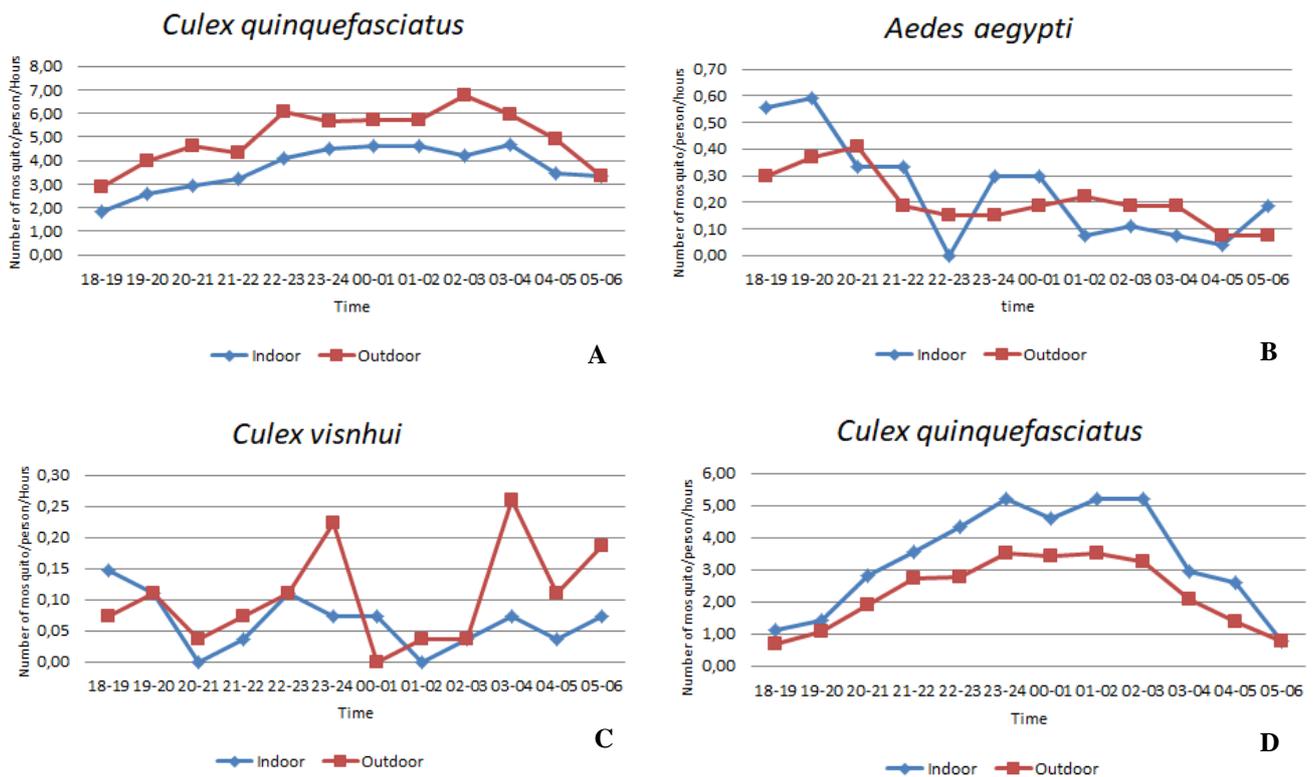


Figure 1. Man Hour Density (MHD) Indoors and Outdoors the Mosquito (A) *Cx. quinquefasciatus*; (B) *Ae. aegypti*; (C). *Cx. vishnui* in Tamansari Village, Rumpin Sub-District; and Mosquito (D) *Cx. quinquefasciatus* in Cimanggis Village, Bojong Gede Sub-District, Bogor District, West Java, Indonesia

Table 5. Mosquito parity, PCR methods and L3 microfilariae larvae detection with dissecting technique against mosquitoes collected during September 2019-February 2020 in Tamansari Village, Rumpin sub-district, Bogor District during September 2019-February 2020

Spesies	n	P	NP	PR (%)	PCR Methods		Dissecting technique	
					n	Positive	n	Positive
<i>Cx. quinquefasciatus</i>	3926	3655	271	93.1	2079	0	1576	0
<i>Cx. vishnui</i>	80	72	8	90	72	0	0	0
<i>Ae. aegypti</i>	229	218	11	95.2	25	0	0	0
<i>Ma. annulata</i>	3	1	2	33.33	199	0	19	0
<i>Cx. tritaeniorhynchus</i>	31	25	6	80.65	14	0	0	0
<i>Ar. kesseli</i>	25	25	0	100	25	0	0	0
<i>Ar. subalbatus</i>	32	30	2	93.75	30	0	0	0
<i>Ae. albopictus</i>	14	14	0	100	1	0	0	0
Total	4340	4040	300		2445	0	1595	0

Note: n: number of mosquitoes; P: Parous; NP: Nully parous; PR: Parity rate

Table 6. Mosquito parity, PCR methods and L3 microfilariae larvae detection with dissecting technique against mosquitoes collected during September 2019-February 2020 in Cimanggis Village, Bojong Gede sub-district, Bogor District, West Java, Indonesia

Spesies	n	P	NP	PR (%)	PCR Methods		Dissecting technique	
					N	Positif	n	Positif
<i>Cx. quinquefasciatus</i>	2628	2429	199	92.43	1357	0	1072	0
<i>Cx. vishnui</i>	9	9	0	100	9	0	0	0
<i>Ae. aegypti</i>	27	27	0	100	4	0	0	0
<i>Cx. tritaeniorhynchus</i>	4	4	0	100	27	0	0	0
<i>Ar. kesseli</i>	44	37	7	84.09	37	0	0	0
<i>Ar. Subalbatus</i>	35	28	7	80	28	0	0	0
Total	2747	2534	213		1462	0	1072	0

Note: n: number of mosquitoes; P: Parous; NP: Nully parous; PR: Parity rate

Mosquito *Cx. quinquefasciatus* generally had more bloodsucking behavior per hour indoors than outdoors in Tamansari Village. Peak density sucking *Cx. quinquefasciatus* indoors occurs at 23.00-24.00 while outdoors occurs at 02.00-03.00. The same situation was found in Cimanggis Village, that the *Cx. quinquefasciatus* suck more blood every hour indoors than outdoors. *Culex quinquefasciatus* peak sucking blood hourly indoors was found at 22.00-23.00, while outdoors was found at 23.00-24.00. This study's results follow the generality that *Cx. quinquefasciatus* was a nocturnal mosquito and nocturnal bite (Dhang et al. 2014) with a time range of 19.00-04.00 (Sukatendra and Shidqon 2016).

Aedes aegypti mosquitoes found sucking blood indoors and outdoors tended to decline. The peak of sucking mosquitoes' blood indoors was at 19.00-20.00, while the peak of sucking the blood outdoors was at 20.00-21.00. According to the theory of bloodsucking activity of *Ae. aegypti* was during the day, especially at 05.00-09.00 am and 3.00-5.00 pm (Kamgang et al. 2012). The results of this study confirmed the nocturnal sucking activity of *Ae. aegypti* in Tamansari Village.

Culex vishnui was generally found to suck more blood outdoors than indoors. Bloodsucking activity *Cx. vishnui* tends to fluctuate. Peak sucking the blood of *Cx. vishnui* outdoors at 03.00-04.00 while indoors at 18.00-19.00. In theory, the activity of *Cx. vishnui* was at night (nocturnal) and peaks above 23.00-00.00 and continues to decline until dawn (Dhang et al. 2014). The abundance of *Cx. vishnui* low visibility indoors led to findings with peak activity at 18.00-19.00, in contrast to the general nature of this mosquito with peak activity above 23.00.

The results of this study confirmed that all mosquito species found had a high paraosity value, which means that the mosquito population in Tamansari Village and Cimanggis Village was very mature at the time of the study. Knowledge of parity status of mosquito infections helps manage and assess infectious diseases mosquitoes on parous were considered potentially infectious (Milali et al. 2020).

Examination of disease agents by dissection methods and PCR on all species of parous mosquitoes that were captured showed no microfilariae were found in the mosquito's body. The number of dissected mosquitoes caught in Tamansari Village was 1595 individuals with a distribution of 1576 *Cx. quinquefasciatus* and 19 *Ae. aegypti*. While the number of mosquitoes carried out by PCR was 2445 in 8 species, with the highest number in *Cx. quinquefasciatus* as many as 2079 individuals and the least number of mosquitoes was *Ma. Annulata*, 1 individual. In Cimanggis Village, 1072 mosquitoes were dissected, all *Cx. quinquefasciatus* while the number of mosquitoes examined by PCR was 1462 in 6 mosquito species, with the highest number in *Cx. quinquefasciatus* as many as 1357 individuals and the smallest number in *Cx. tritaeniorhynchus* as many as 4 individuals. This study is in line with the research of Santoso et al. (2015) in Tanjung Jabung Timur District, which did not find L3 larvae in the surgical method but was different from the PCR

examination found 8 positive samples containing microfilaria DNA.

L3 filarial larvae were not found on examination with the dissection technique, possibly due to the limited ability of mosquitoes to obtain microfilariae when sucking blood. The behavior of microfilariae moves actively to the peripheral blood at certain times, generally at night (nocturnal) which must follow the behavior of mosquitoes when sucking blood. The production of microfilariae must be large enough to spread from one host to another. If only a small number are, only a few mosquitoes can suck microfilariae. However, on the other hand, if too many were inhaled, mosquitoes could die. Other causes could be due to the examiner's skill, the condition of the microfilariae and the condition of the mosquito at the time of surgery. To be easily identified and to find live L3 larvae, the mosquito must be dissected immediately after death. If the microfilariae die, it will be difficult to identify the L3 larvae from the mosquito's body because they were small and thread-like, so they are often covered with faeces from the mosquito's body. The fact that mosquitoes die too long also affects the identification results. Suppose there were L3 larvae in the mosquito's body. In that case, the larvae will die quickly, so it will be very difficult to carry out the identification process.

Detection of microfilariae using the PCR method was faster, more efficient, sensitive and effective than the dissection technique (Plichart and Lemoine 2013). Although the PCR method was more sensitive than the dissection technique, this study did not find any microfilariae in the mosquitoes examined by PCR. This was probably due to the influence of temperature and humidity that can affect the age of the mosquito so that the microfilariae in the mosquito's body do not have time to develop into L3 infective larvae. Another possibility that caused a negative PCR examination was because when the study was taking place, the POPM program was still running, so it was highly suspected that the presence of microfilariae in the patient's body was gone or their production was reduced; as a result, mosquitoes could not suck, or only a few could suck microfilariae.

The absence of detection of microfilariae in the tested mosquitoes should not reduce the awareness of filariasis transmission. The mosquito species collected in endemic areas are reported as the main vectors of this disease in various regions. In addition, due to the small number of mosquitoes and the short catching time, it is necessary to carry out a wider detection scope considering that this disease is endemic in this area.

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