Analysis of Entomological Parameters Using RT-PCR Method The Post-Popm in Simbang Kulon Village, Buaran District, Pekalongan Regency

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Abstract: *Lymphatic filariasis is spread almost throughout Indonesia, some areas are categorized as filariasis endemic areas. The endemicity of filariasis in an area is determined by the microfilariae number (MF rate) above 1% by means of a finger blood survey (SDJ). Simbang Kulon Village in Buaran District, Pekalongan Regency is one of the filariasis endemic areas with an MF rate of 2.65%. This study aims to measure the entomological parameters and molecular examination of the transmission of lymphatic filariasis in Simbang Kulon Village. This study used a survey with a cross-sectional study design. Mosquito catching was carried out using the Human Landing Collection and Resting method both inside and outside the house. The results of the mosquito catching were brought to the laboratory for rearing process for 14 days. Data were analyzed descriptively. The results of the identification of 50 mosquitoes found 94% Culex quinquefasciatus mosquitoes and 6% Culex vishnui mosquitoes. Parous rate, infection rate and infective rate of Culex quinquefasciatus and Culex vishnui were zero per cent. Culex quinquefasciatus had the highest relative abundance (23.5%). Based on the results of the analysis of research data, filaria larvae were not found in the mosquito body because the two Culex mosquito species had never laid eggs (nulliparous).*

Kata Kunci:
Parameter Entomologi, Transmisi Filariasis Limfatik, Kelurahan Simbang Kulon.

Abstrack: Filariasis limfatik tersebar hampir di seluruh Indonesia, beberapa daerah dikategorikan sebagai daerah endemis filariasis. Endemisitas filariasis suatu daerah ditentukan oleh angka mikrofilaria (*MF rate*) di atas 1% melalui pemeriksaan survei darah jari (SDJ). Kelurahan Simbang Kulon di Kecamatan Buaran, Kabupaten Pekalongan merupakan salah satu daerah endemis filariasis dengan *MF rate* 2,65%. Penelitian ini bertujuan mengukur parameter entomologi dan pemeriksaan molekular pada transmisi filariasis limfatik di Kelurahan Simbang Kulon. Penelitian ini menggunakan survei dengan desain studi *cross-sectional*. Penangkapan nyamuk dilakukan dengan metoda *Human Landing Collection* dan *Resting* baik di dalam maupun di luar rumah. Hasil penangkapan nyamuk dibawa ke laboratorium untuk proses *rearing* selama 14 hari. Data dianalisis secara deskriptif. Hasil identifikasi 50 nyamuk didapati 94% nyamuk *Culex quinquefasciatus* dan 6% nyamuk *Culex vishnui*. Tingkat paritas, tingkat infeksi dan tingkat infektif *Culex quinquefasciatus* dan *Culex vishnui* adalah nol persen. *Culex quinquefasciatus* memiliki kelimpahan nisbi tertinggi (23,5%). Berdasarkan hasil analisis data penelitian, larva filaria tidak ditemukan dalam tubuh nyamuk disebabkan kedua spesies nyamuk *Culex* belum pernah bertelur (*nulliparous*).

Introduction

Lymphatic filariasis is a chronic infectious disease caused by filaria worms and transmitted to humans through the bites of various types of mosquitoes (Kemenkes RI, 2019; WHO, 2013). Filaria disease is a neglected tropical disease (NTD) and is still a global health problem because it causes lifelong disability and other social problems (Hussain et al., 2014; Sularno et al., 2017; Welburn et al., 2015). In Indonesia, several regions have cases of filariasis which are high enough to be categorized as filariasis endemic areas (Pratiwi et al., 2019). The endemicity of filariasis in a(Syuhada, Y. et al., 2012)n area is determined by the microfilaria rate (MF rate) above 1% through finger blood surveys (Irawan et al., 2018). One of the

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provinces in Indonesia that is endemic for filariasis is Central Java (Dinas Kesehatan Provinsi Jateng, 2019). Kelurahan Simbang Kulon with a MF rate of 2.65% is one of the filariasis endemic areas in Buaran District, Pekalongan Regency in year 2008 (Syuhada, Y. et al., 2012).

The government through the Ministry of Health of the Republic of Indonesia has implemented a lymphatic filariasis elimination program that implements the "Global Elimination Lymphatic Filariasis" from the WHO to reduce the microfilariae rate to less than 1% in each district/city (Elytha, 2014). Breaking the chain of transmission of filariasis is carried out by means of POPM (Mass Prevention of Drugs/MPD) filariasis and vector control (Kemenkes RI, 2014; Rahanyamtel et al., 2019).

The Pekalongan District Health Office carried out filariasis POPM in 2015-2019 in all areas of Pekalongan Regency as an effort to eliminate filariasis. Evaluation of the fifth year of filariasis POPM implementation in Pekalongan Regency was carried out to determine the average microfilariae achieved after filariasis POPM. The Microfilaria Prevalence Evaluation Survey Activities after filariasis POPM (Pre-TAS) and the Transmission Assessment Survey (TAS 1) which were carried out in 2021 in Pekalongan Regency obtained a microfilariae rate of less than 1% (BBTKLPP Yogyakarta, 2021; Dinas Kesehatan Kabupaten Pekalongan, 2021). The filariasis POPM activity must be supported by two entomological approaches, namely vector control and xenomonitoring/xenosurveillance, which serve to measure the capacity of mosquitoes as vectors of lymphatic filariasis (WHO, 2013).

In Simbang Kulon Village, researches have been carried out using the FBS (Finger Blood Survey) method of filariasis sufferers. Therefore, by considering costs, transportation, orientation in the field of medical entomology and local wisdom, this study conducted a survey of entomological parameters and xenomonitoring to mosquito capacity as a comprehensive vector control measure. The purpose of this study was to measure the entomological parameters and molecular examination of post-POPM lymphatic filariasis transmission in Simbang Kulon Village, Buaran District, Pekalongan Regency.

Research Methods

This study uses a survey with a cross-sectional study design and data analysis using descriptive analysis. The sample was the result of catching mosquitoes in three selected houses around the homes of clinical filariasis sufferers using the Human Landing Collection and Resting method from 18.00 to 24.00 WIB inside and outside the house (Rosanti et al., 2017). The mosquito sample used in this study has received an ethical feasibility certificate with registration number KEPK 223/KEPK/XI/2020. The entomological parameters to be measured are morphologically identifying lymphatic filariasis vector mosquito species, relative abundance, mosquito Parousity level, Infection Rate, measuring Infective Rate using a microscope and molecular examination. This research was conducted in Simbang Kulon Village, Buaran District, Pekalongan Regency and at the Banjarnegara Research and Development Center Laboratory.

Sample Collection Procedure

Mosquito catching activities were carried out simultaneously for three houses with a total of six collectors. The arrests were carried out by two collectors per house with the task of one person collecting mosquitoes inside the house and another person collecting mosquitoes outside the house. Mosquito collection is done every hour.

The activity of catching mosquitoes in the house is carried out by collectors in the living room or in the bedroom. Catching mosquitoes outside the house is done by collectors in places with dim or slightly dark lighting. Collectors sit well, roll up trouser legs to knees and must not smoke. The mosquitoes that were caught were transferred to paper cups and covered with gauze with a hole in the middle for the sugar-water cotton swab as a mosquito feeding container and tied with rubber. Paper cups are labeled by date, time and location of arrest. The catch is brought to the laboratory for rearing for 14 days before identification and surgery are carried out (Handayani et al., 2017; Rahanyamtel et al., 2019; Rosanti et al., 2017).

Results and Discussion

The results of catching mosquitoes in Simbang Kulon Village were found to be 86 mosquitoes (Table 1).

Table 1. Results of mosquito catching in Simbang Kulon Village, Buaran District, Pekalongan Regency in the second week of March 2021

Hour	Mosquito Sample					
	House 1		House 2		House 3	
	I	O	I	O	I	O
18.00 – 19.00	2	-	1	3	3	2
19.00 – 20.00	1	3	4	1	2	5
20.00 – 21.00	1	1	5	3	5	5
21.00 – 22.00	-	-	4	1	4	6
22.00 – 23.00	4	-	4	-	1	2
23.00 – 24.00	1	4	2	1	2	3
TOTAL	9	8	20	9	17	23

Description: I = in; and O = outside

Table 1 shows that in the first house, the mosquito population was more found inside than outside the house. In the second house, the mosquito population was found inside more than outside the house. In the third house, the mosquito population was found outside rather than inside the house. The results of catching mosquitoes were brought to the laboratory for rearing for 14 days with the aim of developing filaria larvae to the L3 phase. The number of mosquitoes that were still alive after being bred for 14 days in the laboratory was found to be 50 mosquitoes. Mosquito death is due to the inability of mosquitoes to survive and be eaten by predators (*ants*).

Mosquito Identification

At the time of identification of the research sample using the Indonesian mosquito picture key book, it was found that the proportion of female *Cx.vishnui* mosquitoes was around three mosquitoes (6%), female *Cx.quinquefasciatus* mosquitoes about 42 mosquitoes (84%) and male *Cx.quinquefasciatus* mosquitoes around five mosquitoes (10%). Due to the greater

number of *Cx.quinquefasciatus* mosquitoes than other mosquitoes, *Culex quinquefasciatus* dominated the diversity of mosquito species in Simbang Kulon Village during the research survey.

Relative Abundance

Analysis of relative abundance was carried out to determine the abundance proportion of certain species (Table 2).

Table 2. The relative abundance of mosquito species in Simbang Kulon Village during the research survey

Species	Amount	Relative Abundance (%)
Cx.vishnui	3	1,5
Cx.quinquefasciatus	47	23,5
TOTAL	50	

Based on Table 2, the relative abundance of *Cx.quinquefasciatus* was greater than the relative abundance of *Cx.vishnui*. This indicates that the proportion of the population of *Culex quinquefasciatus* in the study area is abundant so that it is easy to find and has the opportunity to become a vector of filariasis transmission in Simbang Kulon Village during the research survey.

Parity rate, infection rate and infective rate

The results of infection rate, effective rate and parity rate are in Table 3

Table 3. The level of infection, infectivity and parity of mosquito species in Simbang Kulon Village during the research survey

Species	Amount	*Infection rate*	*Infective rate*	*Parity rate*
Cx.quinquefasciatus female	42	0	0	0
Cx.vishnui female	3	0	0	0
TOTAL	45			

Table 3 shows the parity rate, infection rate and infective rate of zero. This is because all samples of female *Culex* mosquito species are still *nulliparous*, there has been no infection process in the mosquito population as a vector of filariasis or the process of infectivity between mosquitoes as vectors against humans as filariasis hosts during the study.

Molecular Examination

The next analysis was to examine female mosquito samples using Reverse Transcriptase PCR (RT-PCR) to detect the expression of filaria L3 mRNA-activated cuticline transcripts. The samples for RT-PCR were taken from a sample of 45 female mosquitoes which were made into 10 pools consisting of 8 pools containing samples of 5 *Culex quinquefasciatus* mosquitoes, 1 pool containing samples of 2 *Culex quinquefasciatus* mosquitoes and 1 pool containing

samples of 3 *Culex vishnui* mosquitoes. The results of the RT-PCR examination are shown in Table 4 and Figure 1.

Table 4. RT-PCR Examination Results

RT-PCR	Amount	Persentase
Positive	0	0%
Negative	45	100%

Figure 1. RT-PCR examination consists of M: RNA marker ladder 123 bp and S1-10: female mosquito sample pool

Based on Table 4 and Figure 1, RT-PCR examination using Wb-cut-1.2 forward and Wb-cut-1.2 reverse primers with WbL3 target RNA at 123 bp. In Figure 1, it can be seen that the electrophoresis results of samples 1 to 10 did not show any bands, especially at the 123 bp target.

Discussion

The mosquito fauna found in Simbang Kulon Sub-district consisted of one genus, namely the genus *Culex* and two species, namely *Culex quinquefasciatus* and *Culex vishnui* with the largest proportion being *Culex quinquefasciatus* around 42 mosquitoes (84%) (Kemenkes RI, 2011). The morphology of *Culex quinquefasciatus* is as follows: the mosquito is golden brown, the proboscis has no white band, the scutum is covered with evenly distributed brown scales, the pleuron is pale evenly distributed, then on the abdominal tergite there is a white band between the black part of the tergite. The characteristics of *Culex vishnui* are the anterior surface of the dominant midfoot femur is dark in colour, the proboscis has a white band, the scutum is covered with golden yellow scales, the pleuron is evenly brown in colour, then on the abdominal tergite there is a white band (Choirunnisa et al., 2019; Kemenkes RI, 2011).

The diversity of mosquito fauna is highly dependent on local geographical conditions (Kemenkes RI, 2011; Ramadhani & Wahyudi, 2015). Kelurahan Simbang Kulon is an urban area because it has a dense population and most of the people's livelihood is batik so that the dominant mosquito species is *Culex quinquefasciatus* (Rizkiyanti & Harsritanto, 2020). However, the level of diversity of *Cx.quinquefasciatus* mosquitoes found during the research survey was categorized as low. This is because mosquito catching is done during the rainy season. In the rainy season, excessive rainfall can sweep away mosquito breeding grounds so that many eggs, larvae and pupae are carried away by water currents (Handayani et al., 2017).

The species diversity obtained in this study is much lower than the study reported from Serian (Sarawak, Malaysia) which found 27 species and six genera of mosquitoes (Rohani et al., 2013). This low level of faunal diversity was also reported in a study in the Pekalongan region which found two genera and three species, namely *Cx.quinquefasciatus*, *Cx.vishnui* and *Ae.aegypti* (Windiastuti et al., 2013). The difference between the diversity and abundance of mosquito species in the research results in various regions is caused by geographical characteristics, climate (especially temperature and rainfall) and the availability of breeding sites for mosquito species. The local climate has an important role in determining the distribution and diversity of filariasis vectors (Pratiwi et al., 2019).

Culex quinquefasciatus has a great chance of being the main vector of filariasis transmission if filariasis infection occurs, because the proportion of its population is abundant in the environment during the study. This is in line with a study that was reported in Langsa City where *Cx.quinquefasciatus* had a relative abundance of about 18% higher than other mosquito species, which means that this mosquito is the main vector in filariasis transmission (Yulidar et al., 2019). South Kalimantan where *Cx.quinquefasciatus* is the main vector of filariasis transmission because it has the highest relative abundance namely 58.3% (Ridha et al., 2019). Another study also showed a higher relative abundance of *Culex quinquefasciatus* (62.6%) than *An.gambiae* (37.4%) in Jos City, Nigeria (Njunwa & Irving-Bell, 2016).

The relative abundance is strongly influenced by rainfall and inversely proportional to temperature because rain makes breeding sites more available in nature (Uttah et al., 2013). Vector populations that are abundant in nature increase the chances of contact between humans and vectors, so that the risk of transmission increases (Pahlepi et al., 2020).

Based on the results of ovarian surgery, it was found that the parous level of *Cx.quinquefasciatus* and *Cx.vishnui* was zero percent where both species of *Culex* mosquitoes had never laid eggs (*nulliparous*). The picture of the *nulliparous* ovary obtained by microscopy is the size of the mosquito ovary that has not been enlarged and the ends of the tracheolar coils (Njunwa & Irving-Bell, 2016).

The more dominant *nulliparous* proportion is estimated that the captured mosquitoes are newly hatched mosquitoes and the location of their breeding sites is not far from the fishing ground. If an area has a low parity rate, the mosquito population is mostly young in that area (Portunasari et al., 2016). However, it was reported differently from the opinion of other researchers which stated that the proportion of n*ulliparous* female mosquitoes was higher during the dry season and the proportion of parous female mosquitoes was higher in the rainy season (Uttah et al., 2013; Yokoly et al., 2020).

In addition to parity level, examination of mosquito ovaries can also determine the number of gonotrophic cycles experienced by mosquitoes (WHO, 2013). Based on the mosquito life cycle, only female mosquitoes suck blood to mature their eggs (Handayani et al., 2017). Therefore, the higher the proportion of parous mosquitoes, the longer the lifespan and survival mosquitoes so that the greater the chance of transmitting the disease (WHO, 2013).

Parameters that assess the intensity of filariasis transmission in endemic areas include the level of infection (proportion of mosquitoes infected with any stage of filariasis) and infective level (proportion of mosquitoes infected with stage three filaria worms) (Sasa, 1976; WHO, 2013). The values of the infection rate and infective rate of vectors often vary based on seasonal changes in the year in survival rates, such as filaria infection in the human population and the density of filaria larvae infection in the vector population (Sasa, 1976). The results of the microscopic study showed that the ratio of the level of infection and infectivity of filaria larvae

in the body of *Culex* sp mosquitoes was zero percent. This means that the filaria larvae of *W.bancrofti* (L3) were not found in the body of *Culex* sp. The study reported in Pekalongan City showed an infection rate of 0.06% and an infectivity rate of zero percent (Rosanti et al., 2017). The results of other researchers reports also showed an infection rate of about 13.20% and an infectivity rate of about 3.70% in the Assam region, India (Khan et al., 2015).

The results of RT-PCR amplification were negative where there were no filaria larvae of *W.bancrofti* stage III in the mosquito's body. The negative results in this study were due to the parousity level of mosquitoes in the study area found to be *nulliparous*. The results of this study are different from studies that have been reported where there were seven samples (38.89%) that were positive for filaria larval DNA among 18 samples of mosquito DNA isolates (Nasution et al., 2018), but the results of other studies reported that from 184 pools of *An.gambiae* and 152 pools of *Cx.quinquefasciatus*, only three and two pools showed positive DNA *W.bancrofti* (Yokoly et al., 2020).

PCR examination of mosquitoes is used to identify mosquitoes that act as vectors so that they can be used as a basis for vector control to support the elimination of filariasis in areas with high transmission rates (Pahlepi et al., 2020). The selection of the PCR method was based on the sensitivity of PCR which is better than microscopic analysis in detecting availability filaria larvae. Filaria larvae were not found in the mosquito's body, this could be due to the success of the filariasis POPM program at the study site (Pratiwi et al., 2019).

Conclusion

The mosquito species found were *Culex quinquefasciatus* and *Culex vishnui*. The *Cx. quinquefasciatus* mosquito had a dominant relative abundance at the study site. Filaria larvae were not found in the body of *Culex* sp mosquitoes based on the results of the infection rate, infective rate and RT-PCR examination. In this study, because the results of the measurement of the infection rate and the infective level were zero where no filaria larvae were found in the mosquito's body and to strengthen the results of this study, the researcher suggested that an examination using a Finger Blood Survey (SDJ) be carried out to determine the presence or absence of filaria larvae in the body man. Due to limited infrastructure, environmental situation and conditions (covid-19 pandemic) and research funds, this research data was only collected overnight.

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