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Detection of microfilaria L3 and insecticide resistance among wild-caught mosquito vectors in endemic areas of lymphatic filariasis

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Abstract. *Ghofur A, Hadisaputro S, Sayono S. 2024. Detection of microfilaria L3 and insecticide resistance among wild-caught mosquito vectors in endemic areas of lymphatic filariasis. Biodiversitas 25: 1975-1983.* The decline in the global prevalence of Lymphatic Filariasis (LF) is evident in the persistent endemic foci in Africa and Southeast Asia. In this context, the presence of infectious and insecticide-resistant mosquitoes and the annual biting rate in the area are key to microfilaria transmission. Therefore, this research aimed to determine insecticide resistance and microfilariae infection in mosquito vectors in endemic areas. In Jenggot and Medono Villages, twice entomological surveys were conducted based on six LF cases, where indoor and outdoor mosquito capturing was carried out in ten houses within a 100 m radius of each case as well as household interviews about insecticide use. In addition, laboratory works were performed for species identification and detection of ovarian dilatation, microfilariae infection, and knockdown resistance mutations. A total of 1,197 and 581 mosquitoes were distributed to five species and their proportions, namely *Culex quinquefasciatus* (69.59 and 65.40%), *Cx. tritaeniorhynchus* (5.76 and 0.00%), *Cx. vishnui* (5.85 and 27.54%), *Anopheles vagus* (0.58 and 0.00%), and *Aedes aegypti* (18.21 and 7.06%). The percentage of female mosquito infection, parity, and microfilaria were 66.25 and 86.00%, 63.37 and 70.95%, as well as 0.00 and 1.34%, respectively. Furthermore, TTA-TTT, TTA-CTA, and TTA-TGT base substitutions were reported in codon 1014 of *Cx. quinquefasciatus* VGSC gene with proportions of 81.66, 1.67, and 26.67%, respectively. The result showed that the vulnerability of the research location to transmission emphasized the necessity for early detection, vector control, and further analyses of the susceptibility of microfilariae to antiparasitic drugs.

Keywords: *Culex quinquefasciatus*, microscopic, molecular detection, mosquito vectors, *Wuchereria bancrofti*

INTRODUCTION

The decline in the global prevalence of Lymphatic Filariasis (LF) is evident in the persistent endemic foci in Africa and Southeast Asia (NTD Collaborators 2020). The prevalence of LF in Asia is stable at 3%, and only four countries are free of the disease, namely China, Japan, Vietnam, and South Korea (Bizhani et al. 2021). Despite six rounds of mass drug administration (MDA), the persistence of microfilaria infections continues, a situation exemplified by Myanmar (Dickson et al. 2018). This low decrease in LF prevalence after Preventive chemotherapy, Operations, Monitoring, and Participation (POMP) in endemic areas is reported to be due to a lack of community understanding and participation in the efforts (Aboagye and Addison 2022). Other research reports that the transmission is influenced by high mosquito vector density due to favorable rainfall, temperature, and air humidity (Sinha et al. 2023), resulting in the proportion of infectious vectors (Dharmarajan et al. 2019; Davis et al. 2019).

Data and information on competent vectors and the circulation of microfilariae in mosquitoes are needed to complete MDA in endemic areas by prioritizing vector mosquito control efforts. However, this data and information is very limited in Indonesia, specifically in Central Java. The condition requires further research to understand the interaction of pathogens with mosquitoes as input. This strengthens methods and strategies for controlling LF vector mosquitoes (Famakinde 2018), including developing predictive models of risk factors for LF transmission and public health interventions (Zerbo et al. 2021). Furthermore, the results of entomological surveys in several countries report varying results. Research in Mafia Island, Tanzania resulted in three mosquito species of LF vector, namely *Cx. quinquefasciatus*, *Anopheles gambiae*, and *An. funestus*, and 0.25% of the examined mosquitoes were infected with *W. bancrofti* (Derua et al. 2017). In Masasi District, Tanzania, the research found the same three species with a higher *W. bancrofti* infection rate for *Cx. quinquefasciatus* (Lupenza et al. 2021). Research in Bogor District (West Java) reported five mosquito genera, namely *Culex*, *Mansonia*, *Aedes*, and *Armigeres*, without detecting microfilariae (Nirwan et al. 2022). Another research in Pekalongan District (Central Java) captured three mosquito species, namely *Cx. quinquefasciatus*, *Cx. vishnui*, and *Ae. aegypti*, and 0.43% of samples were positive for microfilaria (Nurjazuli et al. 2022). The results showed a variation in the vectors and the infection rates of microfilaria in different regions.

The burden of LF vector control efforts is compounded by the resistance of *Cx. quinquefasciatus* to insecticides as reported in Bengal, India (Rai et al. 2019) and Uganda (Silva-Martins et al. 2019). Resistance of *Cx. quinquefasciatus* mosquitoes to insecticide classes, deltamethrine 0.05% and temephos 0.75% occur widely in Sri Lanka accompanied by the L1014F knockdown (kdr) mutation (Chandrasiri et al. 2020). The L1014S and L1014F mutations were also reported in *Cx. pipiens pallens* mosquito in China (Liu et al. 2019). Resistance to *Cx. quinquefasciatus* was also reported from Grobogan and Pekalongan in Central Java to 0.75% permethrin (Chakim et al. 2017) based on bioassay tests. Therefore, this research aimed to determine the biological characteristics, insecticide resistance, and the presence of microfilariae in mosquito vectors in LF endemic areas of Pekalongan City, Central Java Province, Indonesia.

MATERIALS AND METHODS

Study sites

As many as 20 sites in Jenggot and 9 sites in Medono Villages were selected as research locations based on filariasis case surveillance data from the Pekalongan City Health Service in 2020 of the Central Java Province, Indonesia (Figure 1). Furthermore, Jenggot Village possessed the highest microfilaria rate (5.4%) in Pekalongan City and five cases were detected in 2020. Medono Village was not an endemic area for LF, but two new filariasis sufferers were found in a finger blood survey in 2020 (Dinas Kesehatan Kota Pekalongan 2021). This cross-sectional research included six cases and 10 houses around the case within a radius of 100 m.

Ethical clearance, data collection and analysis

Data collection was conducted after the ethical review was achieved. This research has received a letter of recommendation with the issuance of ethical clearance number: 419/EA/KEPK-FKM/2021 from the health ethics committee KEPK FKM Universitas Diponegoro Semarang. Furthermore, twice entomological and household survey was conducted in Jenggot and Medono Villages. The data collected were analyzed to describe each research variable in the form of tables, pictures, and maps.

Mosquito catching procedures

Mosquito catching was carried out at night from 18.00-06.00 at the house of the new LF case and 9-10 neighboring houses within a 100 m radius according to standard procedures (WHO 2013). As many as 6 people were trained to apply the Human Landing Catch (HLC) and Resting Collection (RC) methods. In general, the HLC method involved the mosquito catcher carrying an aspirator and sitting at the capture location with one leg up to the calf open. Mosquitoes that land on their feet are sucked in using an aspirator. In contrast to HLC, the target of the RC method is mosquitoes that land on the walls of the house. The team was divided into two to catch mosquitoes inside and outside the house for 40 and 10 minutes to change the paper cup. Every 1 hour, the mosquitoes are put in a paper cup with a code and time of capture. RC was carried out from 06.00-07.30 to catch samples resting on walls, window curtains, mosquito nets, and hanging clothes, using an aspirator and net. The mosquitoes caught are put into a paper cup filled with cotton filled with sugar solution. The paper cup is placed in a container covered with banana stems and a wet towel to maintain optimum temperature and humidity of 27±2° C and 80±10%, respectively. The captured mosquito samples were held for twelve days and fed a 10% sugar solution before examination.

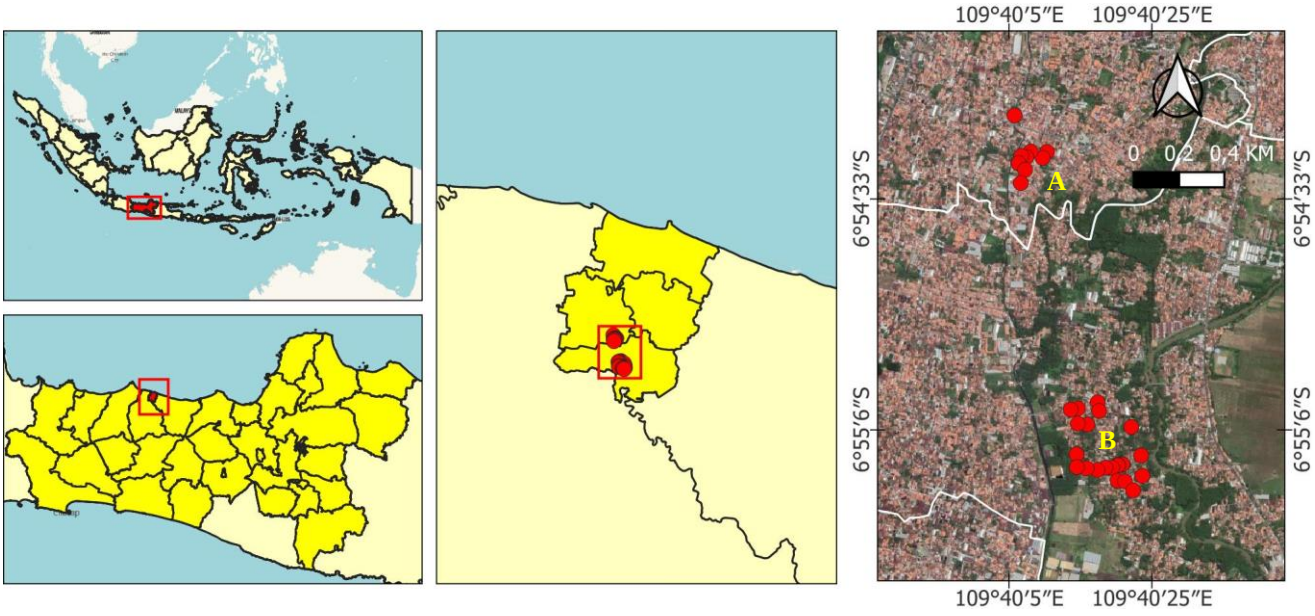

Figure 1. The map of research sites in Pekalongan City, Central Java Province, Indonesia. A. Medono and B. Jenggot

Identification of mosquito species and ovary dilatation

Mosquito species were identified using previously published identification keys (Rattanarithikul et al. 2005; Nugroho and Mujiyono 2021). The porosity was determined using a surgical method to determine the amount of ovarian dilatation. The identified mosquito is carefully positioned within a petri dish, where the wings and legs are separated from the body. The specimen is delicately positioned onto a glass apparatus, where physiological NaCl solution is precisely administered. Additionally, surgery is performed using a surgical needle and carried out using a stereo microscope. The surgical needle in the left and right hands presses the chest and the 7th segment. The ovaries were placed on a glass object given distilled water to view the tracheoles using a stereo microscope with a magnification of 40x10.

Microfilaria detection

The wings of the mosquito slated for dissection were incised with precision to prevent the dispersion of scales, ensuring the integrity of the microscope's field of view. Mosquitoes were placed on glass slides dripped with physiological saline. The thorax and abdomen were cut into pieces with a dissecting needle and observed under a microscope at 40X magnification. Meanwhile, the presence of filarial worms appears to move depending on the stage. In this context, stages 1-2 are short, fat, and slow-moving, while stage 3 appears long and fast-moving (Laney et al. 2010). Microfilaria detection also uses molecular methods with the stages of DNA extraction, amplification, electrophoresis, and imaging.

DNA extraction

A total of 10-20 female mosquitoes from each location were pooled based on species. Each pool was homogenized with a pestle in a microcentrifuge tube containing 180 µL of ATL buffer (pH 7.2; PBS) and ground, 20 µL of proteinase K, vortexed, and incubated for 24 hours. In the subsequent step, the specimen was vortexed for 15 seconds, and 200 µL AL buffer was added. A total of 600 µL of extract samples were put into a mini-column and centrifuged for 1 minute at a speed of 8000 rpm. Approximately 500 µL of Aw1 was added to the specimen, and centrifuged for 1 minute, before adding 500 µL of AW2 buffer and centrifuged at 14,000 rpm for 3 minutes. The sample was transferred to a 1.5 ml tube and 60 µL AE buffer was added, incubated for 1 minute, and centrifuged at 8000 rpm for 1 minute.

DNA amplification with polymerase-chained reaction

The extracted mosquito DNA was amplified using a thermal cycler (Perkin-Elmer Cetus, Norwalk, Connecticut, USA) with 2 oligonucleotide primers, NV-l: 5' CGTGATGGCATCAAAGTAGCG 3' (21-mer) and NV-2: 5'CCCTCACTTACCATAAGACAAC 3' (22- mer). Each amplification reaction was carried out in a final volume of 50 and contained 10 ~ I-IMT ri-HCl pH 9.2, 1.5 mM MgCl, 75 mM KCl, 1.25 mM each deoxy-nucleotide triphosphate, 10 pmol each primer NV-l and NV -2, and 2 units of Taq polymerase. Furthermore, the temperature program for PCR was 5 minutes at 95° C, followed by 35 cycles of 1 minute each at 94, 55, and 72° C, and an elongation of 10 minutes at 72° C. A total of 20% of the PCR product from each sample was electrophoresed on a 2% agarose gel and stained with ethidium bromide to confirm amplification (Ramzy et al. 1997).

RESULTS AND DISCUSSION

A total of 1,678 mosquitoes were obtained in two capture shifts, namely 1,197 and 581, with a proportion of female sex and parity of 66.25 and 86.00% as well as 63.37 and 70.95%. The majority (61.49 and 65.72%) of mosquitoes showed ≥4 ovarian dilatations or a lifespan of 12-16 days, which is an estimate based on the understanding that a gonotropic cycle is approximately 3-4 days (Fereda 2022). This research applied the mosquito-catching method with HLC and resting catch to obtain a high composition of female mosquitoes and porosity. Gravid female mosquitoes suck blood to meet the protein needs for the development of embryos in eggs. In tropical regions, blood-feeding behavior persists year-round, but in temperate regions, gravid mosquitoes are breeded only during the spring season (Siperstein et al. 2023). Therefore, air temperature influences mosquitoes in their mating and pregnancy behavior. A total of five species were found in the first fishing period, and only three were reported in the second period with the proportion being *Cx. quinquefasciatus* (69.59 and 65.40%), *Cx. tritaeniorhynchus* (5.76 and 0.00%), *Cx. vishnui* (5.85 and 27.54%), *An. vagus* (0.58 and 0.00%), and *Ae. aegypti* (18.21 and 7.06%). The proportion of female mosquitoes in the first and second captures based on species is *Cx. quinquefasciatus* (60.78 and 59.78%), *Cx. tritaeniorhynchus* (8.70 and 0.00%), *Cx. vishnui* (8.83 and 32.06%), *An. Vagus* (0.88 and 0.00%), and *Ae. aegypti* (20.81 and 8.22%) (Tables 1 and 3). These data show that *Cx quinquefasciatus* is the dominant species in the locations, while *Cx. tritaeniorhynchus* and *An. vagus* was found on the second shift of mosquito catching. The dominance of *Cx. quinquefasciatus* in LF endemic areas was also reported in several research such as in Tanzania (Derua et al. 2017; Lupenza et al. 2021), and Gamapaha, Sri Lanka (Pilagolla and Amarasinghe 2021). Similar findings were reported in Bogor Regency, West Java (Nirwan et al. 2022), Pekalongan Regency, Central Java (Nurjazuli et al. 2022), and Tangerang (Prasetyowati et al. 2019). However, several research report different dominant vector species of LF in endemic areas such as *Armigeres subalbatus* in Musi Rawas, South Sumatra (Mulyaningsih et al. 2019), *Ae. scutellaris* and *Ae. kochi* in the South Pacific, as well as *An. gambiae*, *A. funestus*, and *An. punctulatus* in rural Asia and Africa (Bhuvaneswari et al. 2023). Differences in species dominance are influenced by habitat conditions, specifically the presence of aquatic plants (Pratiwi et al. 2019), chloride content, and water temperature (Amini et al. 2020).

Table 1. Distribution of mosquitos based on characteristics and research sites (1st period of mosquito capture)

Variables	Research sites			
	Medono		Jenggot	
	n	%	n	%
Mosquito species and sex				
Cx. quinquefasciatus	280	33.61	553	67.39
Male	124	44.39	227	41.09
Female	156	55.61	326	58.91
Cx. tritaeniorhynchus	31	44.93	38	55.07
Male	0	0.00	0	0.00
Female	31	100.00	38	100.00
Cx. vishnui	29	41.43	41	58.57
Male	0	0.00	0	0.00
Female	29	100.00	41	100.00
An. vagus	0	0.00	7	100.00
Male	0	0.00	0	0.00
Female	0	0.00	7	100.00
Ae. aegypti	83	38.07	135	61.93
Male	17	20.62	36	26.67
Female	66	79.38	99	73.33
Parity	103	33.99	200	66.01
Nulliparous	23	22.33	88	44.00
Parous	80	77.67	112	56.00
No. of ovary dilatation (days)	80	41.67	112	58.33
1 (4)	0	0.00	8	7.14
2 (8)	5	6.25	14	12.50
3 (12)	18	22.50	29	25.89
4 (16)	14	20.48	7	6.25
5 (20)	18	22.50	10	8.93
6 (24)	6	7.50	26	23.21
7 (28)	19	23.75	18	16.07

Table 2. Results of microscopic microfilariae detection of female mosquitoes (1st period of mosquito capture)

Species	Research sites			
	Medono		Jenggot	
	n	%	n	%
Cx. quinquefasciatus	50	50.00	50	50.00
Positive	0	0.00	0	0.00
Negative	50	100.00	50	100.00
Cx. tritaeniorhynchus	10	50.00	10	50.00
Positive	0	0.00	0	0.00
Negative	10	100.00	10	100.00
Cx. vishnui	10	50.00	10	50.00
Positive	0	0.00	0	0.00
Negative	10	100.00	10	100.00
An. vagus	-	-	7	100.00
Positive	-	-	0	00.00
Negative	-	-	7	100.00
Ae. aegypti	10	50.00	10	50.00
Positive	0	0.00	0	00.00
Negative	10	100.00	10	100.00

The results of catching mosquitoes showed different results, where the first period produced greater numbers. This shows that mosquito abundance varies over time but does not correlate with microfilaria findings. The surgery and PCR examination results of mosquito samples from the first capture period did not report microfilariae (Table 2). In the second period of capture, fewer numbers and species were obtained, namely 581 mosquitoes (Table 3). The results of dissection after holding for 12 days found that 4 out of 298 (1.34%) mosquitoes contained L3 microfilariae, with species *W. bancrofti* (Figure 2). The microfilariae were detected from mosquito samples aged 12-20 days (ovarian dilatation 4 and 5) with proportions of 25 and 75%. Therefore, microfilariae are found in mosquitoes subjected to 4-5 periods of sucking blood. Detection of microfilariae in mosquito vectors in Indonesia is still limited. There is still an infectious vector of LF in Pekalongan City, Central Java, even with a higher percentage compared to findings in other areas (Derua et al. 2017; Lupenza et al. 2021; Nurjazuli et al. 2022). In addition, the area still has a potential for LF transmission with the main vector being the *Cx. quinquefasciatus*. Apart from additional data on the number of microfilaria infections in mosquito vectors, the findings also confirm the role of *Cx. quinquefascitus* as the dominance vector of LF in Pekalongan. *W. bancrofti* is the microfilariae most frequently found in mosquito vectors (Kinyatta et al. 2023), specifically *Cx. quinquefasciatus*. The finding also follows similar reports in Tanzania where the species is the main vector of LF (Lupenza et al. 2021). The variation in species and proportion of mosquitoes is also similar to other reports where *Cx. quinquefasciatus* is the dominant species in LF endemic areas (Derua et al. 2017; Lupenza et al. 2021). In the local context, this research found more mosquito species than previous findings at other nearby locations (Nurjazuli et al. 2022). The findings show the high potential for LF transmission in the region. The majority of *Cx. quinquefasciatus* is a female with a high proportion of parity and an estimated lifespan of more than 12 days. These findings show a higher proportion of mosquitoes with ovarian dilatations ≥4 than the number. The data reports that the majority of female *Culex* mosquitoes have a high potential to become competent vectors when in the first or second gonotrophic cycle to obtain microfilariae from the blood of parasitemia sufferers. The probability is supported by the observation that all L3 microfilariae originated from mosquito samples showing an ovarian dilation range of 4-5 times or 12-20 days old. This discovery is consistent with previous findings showing an extrinsic incubation period of 13 days for microfilariae within the body to transition to the L3 stage and migrate to the salivary glands (Xu et al. 2018; Dharmarajan et al. 2019). The potential for LF transmission is increasing with climate change, where the average daily air temperature shortens the extrinsic incubation period for microfilariae (Simon et al. 2017).

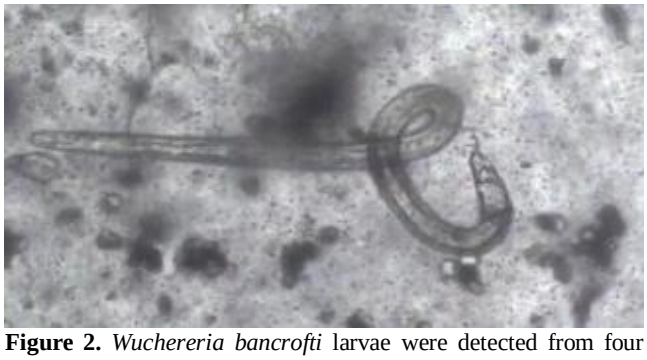

Figure 2. *Wuchereria bancrofti* larvae were detected from four mosquito samples of *Cx. quinquefasciatus* collected from Jenggot village, Pekalongan City

The results of nucleotide substitution analysis conducted on the ace1 and *Cx. quinquefasciatus* VGSC genes serve as indicative markers for assessing the insecticide resistance status within the mosquito population. Part (A) in Figure 3 shows the results of nucleotide consistency at codon 119 of the ace1 gene where DNA samples show the same wildtype base arrangement, namely GGC (Valine amino acid). Meanwhile, part (B) is a chromatogram analysis of the base arrangement. Part (C) is the results of consistency in the nucleotide sequence in codon 1014 of the VGSC gene where there are three forms of nucleotide substitution from T to C, T to G, and A to T in the first, second base, and third bases, respectively. There are three nucleotide substitution variations in codon 1014 of the VGSC gene of *Cx. quinquefasciatus* from the formation of wild-type leucine (TTA) to phenylalanine (TTT) and Cysteine (TGT), as well as a silent mutation from TTA to CTA (Table 4). The first change is a form of silent mutation where TTA to CTA does not change the amino acid, namely Leucine. The second change results in an amino acid change from Leucine (TTA) to Cysteine (TGT) while the third leads to a change in Phenylalanine (TTT) (Figure 2). The L1014F and L1014C mutations (Figure 4) show homozygous and heterozygous forms. Parts A and B are homozygous and heterozygous forms of changing Leucine (TTA) to Phenylalanine (TTT) and Cysteine (TGT), while C and D are homozygous and heterozygous forms of changing Leucine (TTA) to Cysteine (TGT). Image B is a heterozygous form where T and G bases are at the same locus but T is more dominant. The mutation analysis of the VGSC codon 1014 gene shows that the proportion of mosquitoes resistant to insecticides in the population is 98-100%. The utilization of insecticides remains prevalent, with rates ranging from 85 to 92.5%, and some families (63.3-65.0%) still use it for more than two years. Moreover, daily usage intensity ranges from 40 to 53.3%, with particular emphasis on the pyrethroid group at 80 to 81.7% as well as burnt coil formulations, which account for 58 to 60% of usage (Table 5). Reports of resistance to Culex mosquito species, specifically *Cx. quinquefasciatus* is still limited. This finding complements previous data where *Cx. quinquefasciatus* in Central Java has been resistant to pyrethroid insecticides (Chakim et al. 2017). However, research in Jakarta reported that the species was still susceptible to pyrethroids and organophosphates (Subahar et al. 2022). According to recent data, *Cx. quinquefasciatus* in Surabaya has been resistant to both groups of insecticides with mutations in the VGSC and ace-1 genes (Panjinegara et al. 2022). Therefore, Culex mosquito resistance to insecticides has become increasingly widespread. The phenomenon of expanding the resistance area of *Cx quinquefasciatus* was also reported in Bengal, India (Viswan et al. 2020; Rai et al. 2019; Cameroon (Talipouo et al. 2021), Brazil (Lopes et al. 2019), Nigeria (Omotayo et al. 2023), and Korea (Jeon et al. 2024). Molecular analysis of mosquito samples showed four genotypes of the VGSC gene, namely wild type, silent mutation, and mutant (Leu-Phe and Leu-Cis). The Leu-Phe mutation is the most frequently reported mutant form in Indonesia (Panjinegara et al. 2024) and other countries (Talipouo et al. 2021). This is different from the findings in Surabaya which reports wildtype, mutant-heterozygous, and mutant-homozygous genetic variations (Panjinegara et al. 2024). A serious genetic mutation was reported in Brazil including esterase, ace-1, and VGSC (Lopes et al. 2019). Research in Korea did not obtain the G119S mutation but reported heterozygous forms of AGC/GGC, L1014F, and L1014S (Jeon et al. 2024). This genetic mutation phenomenon shows a serious problem affecting vector control efforts in LF-endemic areas.

Table 3. Distribution of Mosquito Characteristics from Jenggot Village (2nd Period of Mosquito Capture)

Characteristics of mosquitoes	n	%
Mosquito species and sex		
Cx. quinquefasciatus	380	65.40
Male	82	21.58
Female	298	78.42
Cx. vishnui	160	27.54
Male	0	0.00
Female	160	100.00
Ae. aegypti	41	7.06
Male	0	0.00
Female	41	100.00
Total	581	100.00
Parity		
Nulliparous	86	29.05
Parous	210	70.95
Total	296	33.99
No. of ovary dilatation (age of mosquito; days)		
1 (4)	7	3.33
2 (8)	18	8.57
3 (12)	47	22.38
4 (16)	43	20.48
5 (20)	47	22.38
6 (24)	23	10/95
7 (28)	25	11.90
Total	210	100.00
Microscopic detection of microfilariae in female mosquitoes		
Cx. quinquefasciatus	298	59.72
Positive	4	1.34
Negative	294	98.66
Cx. vishnui	160	32.06
Positive	0	0.00
Negative	160	100.00
Ae. aegypti	41	8.22
Positive	0	0.00
Negative	41	100.00
Total	499	100.00

Table 4. Frequency of VGSC gene mutations among *Cx. quinquefasciatus* mosquito

Nucleotide substitution	Type of mutation			
	Homozygous	%	Heterozygous	%
TTA to TTT	33	55.00	16	26.67
TTA to CTA	1	1.67	0	0.00
TTA to TGT	4	6.67	6	10.00

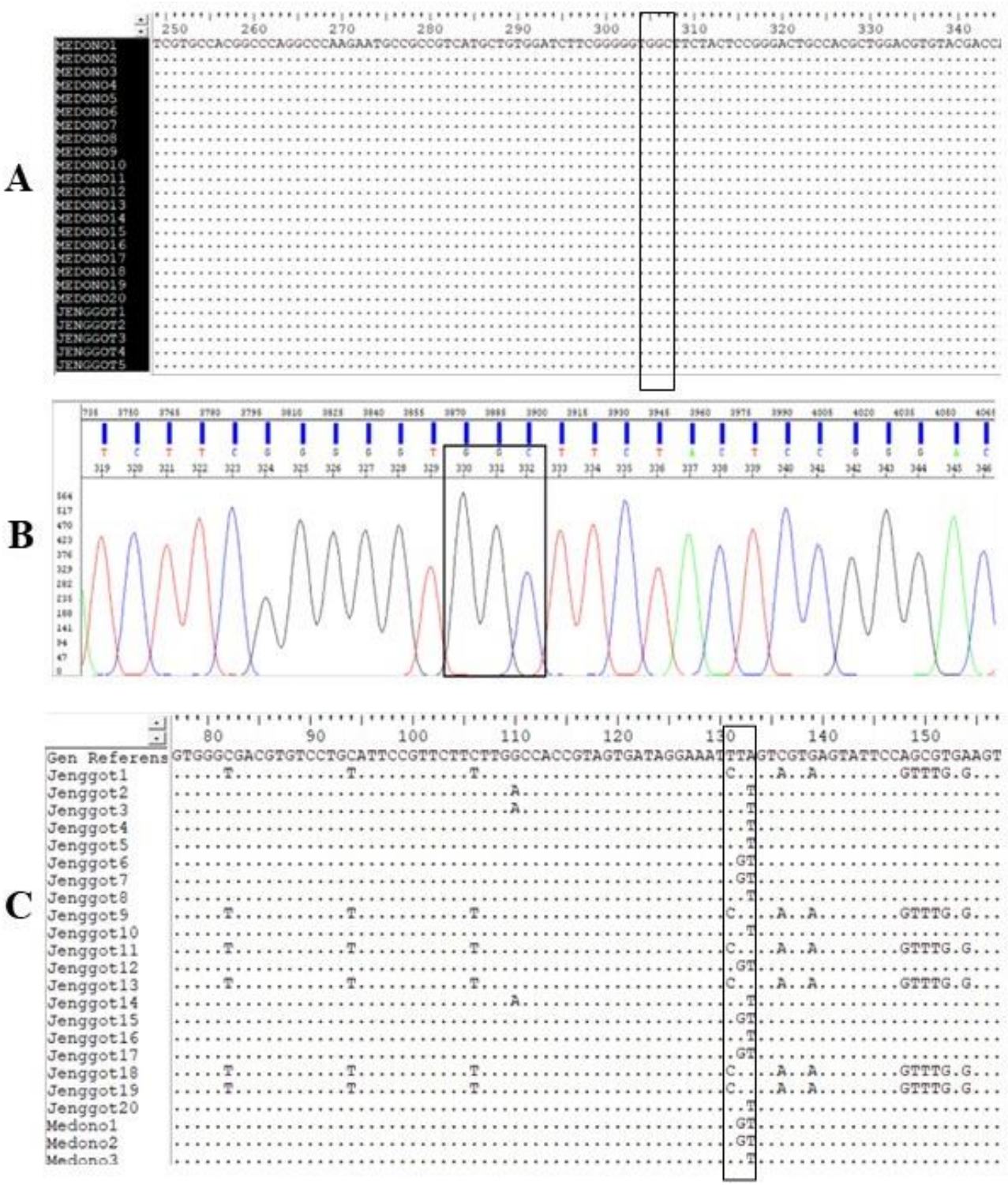

Figure 3. Results of nucleotide substitution analysis of the ace1 and VGSC gene of *Cx. quinquefasciatus* as an indication of the insecticide resistance status of the mosquito population. No nucleotide substitutions were found in codon 119 of the ace1 gene (A) and the chromatogram shape of codon 119 (B). Part C is a variation of nucleotide substitution in codon 1014 of the VGSC gene which shows three variant changes, namely TTA-CTA (silent mutation), TTA-TTT, and TTA-TGT

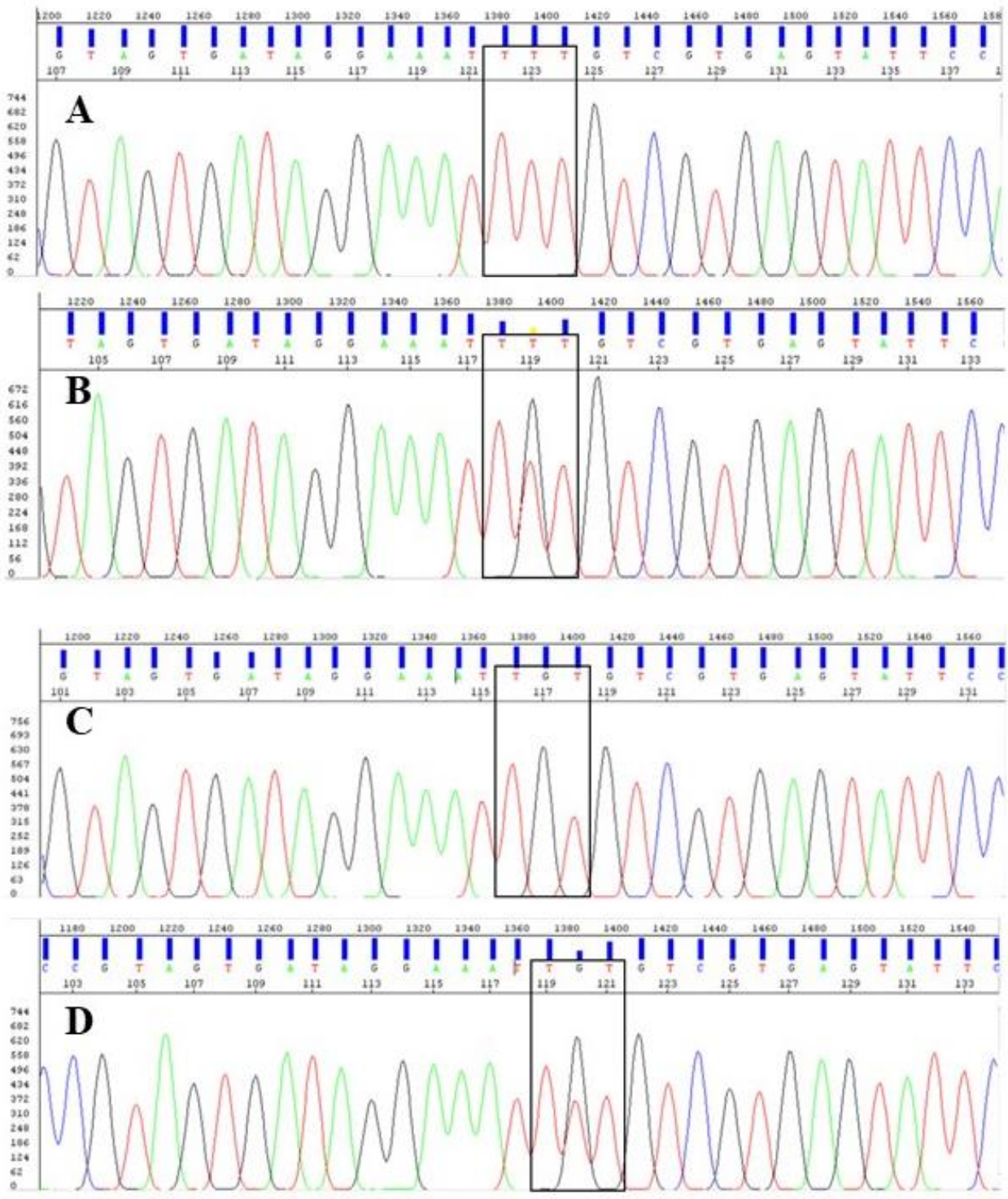

Figure 4. Homozygous and heterozygous nucleotide substitution in codon 1014 of VGSC gene of *Cx. quinquefasciatus*. A and B are homozygous and heterozygous forms of changing the amino acid Leucine (TTA) to Phenylalanine (TTT) and Cysteine (TGT), while C and D are homozygous and heterozygous forms of changing the amino acid Leucine (TTA) to Cysteine (TGT). Image B is a heterozygous form where the bases T and G are at the same locus, although T is more dominant. The opposite condition occurs in image D

Table 5. The insecticide resistance status and history of insecticide use in research sites

Variable	Research sites			
	Medono		Jenggot	
	n	%	n	%
Resistance status				
Sensitive	0	0	1	2
Resistance	20	100	59	98
The use of household insecticide				
Yes	37	92.50	51	85
No	3	7.50	9	15
Insecticide group				
Organophosphate	8	20	11	18.33
Pyrethroid	32	80	49	81.67
History of insecticide use (years)				
< 2	14	35	22	36.67
≥ 2	26	65	38	63.33
Insecticide use intensity				
Not everyday	24	60	28	46.67
Everyday	16	40	32	53.33
Insecticide formulation				
Coil	24	60	35	58.33
Spray	6	15	10	16.67
Electric	2	5	4	6.67
Repellent	8	20	11	18.33

In conclusion, *Cx. quinquefasciatus* was the dominant species among the five vector mosquitos in the LF endemic area of Pekalongan District, and 1.34% were proven to carry *W. bancrofti* microfilariae. Therefore, the area was vulnerable to LF transmission and the condition was increased by *Cx. quinquefasciatus* resistance against pyrethroid and organophosphate class insecticides. Molecular analysis of the VGSC gene found the wild-type allele and three mutant alleles, namely TTA to CTA, TTA to TTT, and TTA to TGT mutations. Further investigation should be conducted to detect the susceptibility of microfilariae to various antiparasitic drugs and educate the public in implementing methods of self-protection from exposure to mosquitoes accompanied by environmental cleaning movements.

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