BIODIVERSITAS
Volume 25, Number 9, September 2024
Pages: 3125-3131

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250934

Species diversity, mosquito behavior, and microfilariae detection in vectors and reservoirs in filariasis-endemic areas of Bengkulu, Indonesia

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Manuscript received: 6 August 2024. Revision accepted: 18 September 2024.

Abstract. *Kermelita D, Hadi UK, Soviana S, Tiuria R, Novianto D. 2024. Species diversity, mosquito behavior, and microfilariae detection in vectors and reservoirs in filariasis-endemic areas of Bengkulu, Indonesia. Biodiversitas 25: 3125-3131.* Filariasis, recognized by the WHO as a neglected tropical disease, remains a significant global health challenge, particularly in Southeast Asia. In Bengkulu Province, Indonesia, 66 cases of filariasis were reported between 2010 and 2020, with the highest prevalence found in Mukomuko and Seluma Districts**.** This study analyzed the vector behavior and detection of filariasis reservoirs in endemic areas of Bengkulu Province. Human landing and resting collections were employed for mosquito collection for 12 h (18:00-06:00), and blood sampling was performed on reservoir animals (*Canis familiaris, Felis catus* Linnaeus, 1758, and *Macaca fascicularis* (Raffles, 1821)). Mosquito diversity was analyzed using several diversity indices. Filaria larvae in mosquitoes were detected using Polymerase Chain Reaction (PCR) methods, and filaria in reservoir animals were detected using blood smears with Giemsa staining. The result showed that 17 mosquito species were captured in the filariasis-endemic regions of Bengkulu Province: *Culex quinquefasciatus* Say, 1823; *Culex hutchinsoni* Barraud, 1924; *Culex gelidus* Theobald, 1901; *Culex pseudovishnui* Colless, 1957; *Culex vishnui* Theobald, 1901; *Armigeres subalbatus* (Coquillett, 1898); *Aedes aegypti* (Linnaeus, 1762); *Aedes albopictus* (Skuse, 1894); *Anopheles tessellatus* Theobald, 1901; *Anopheles vagus* Döntiz, 1902; *Anopheles minimus* Theobald, 1901; *Anopheles indefinitus* (Ludlow, 1904); *Anopheles sundaicus* (Rodenwaldt, 1948); *Anopheles barbirostris* Wulp, 1884; *Anopheles maculatus* Theobald, 1901; *Anopheles* sp., and *Mansonia annulata* Leicester, 1908. *Culex pseudovishnui* exhibited the highest indoor density among the mosquito species, whereas *C. quinquefasciatus* exhibited the highest outdoor density. Filaria larvae were not detected in all mosquito sample pools based on the PCR results, whereas microfilariae of *Dirofilaria immitis* (Leidy, 1856) Railliet & Henry, 1911 were identified in the reservoir (Canine) blood samples. Thus, the presence of microfilariae in reservoir animals must be monitored because they can transmit filariasis to the community. Control strategies should be developed for endemic regions in Bengkulu Province.

Keywords: Detection microfilaria, *Dirofilaria immitis*, neglected tropical diseases

INTRODUCTION

Filariasis is caused by filarial nematodes and is transmitted through mosquitoes (Kinyatta et al. 2023). This disease has varied clinical symptoms, including swelling of the legs, and arms, enlarged lymph nodes, pain, fever, fatigue, and enlarged testicles or scrotum in infected men (Kinyatta et al. 2023). The symptoms may progress over time and lead to severe complications depending on the stage of infection and the etiological filarial parasite (Lourens and Ferrell 2019).

The World Health Organization categorizes filariasis as a neglected tropical disease and remains a global health concern (Vadivalagan et al. 2017). Approximately 120 million people in 83 countries are reported to have been infected with filariasis, with the highest prevalence occurring in tropical and subtropical regions, particularly in Southeast Asia (NTD Modelling Consortium Lymphatic Filariasis Group 2019). In total, 8,742 chronic filariasis cases were reported in 34 provinces in 2022, with Bengkulu Province reporting 66 cases (Ministry of Health 2023). Bengkulu Province comprises one city and nine districts, of which five are endemic to filariasis (Ministry of Health 2023). A total of 66 patients with filariasis were reported in Bengkulu Province from 2010-2020, including 32 new cases (Ministry of Health 2023). The highest filariasis cases were in the Mukomuko and Seluma Districts, with 25 and 15 cases, respectively (Ministry of Health 2023). The species of filariasis generally found in Bengkulu Province is *Brugia malayi* (Ministry of Health 2023). However, specific data on the filariasis species present in Mukomuko and Seluma Districts are not available.

Epidemiological transmission of filariasis is influenced by various factors, including the agent, host, vector, environment, and reservoir. Ariati et al. (2020) reported the detection of *Wuchereria bancrofti* (Cobbold, 1877) and *B. malayi* in several mosquito species in several districts in Indonesia following the Provision of Mass Preventive Medicine (POPM). Detection of *B. malayi* in mosquitoes in Indonesia was also reported in Belitung District, Indonesia (Supali et al. 2023), Hulu Sungai Utara and West Kotawaringin Districts (Andiarsa et al. 2018; Rahayu et al. 2019), and Balangan District (Supriyono and Tan 2020). Similar studies have reported the detection of *W. bancrofti*

in mosquitoes in Tangerang (Nasution et al. 2018), Pekalongan, Lebak in Java (Lee and Ryu 2019), Aceh Jaya, Pesisir Selatan, Bangka Barat, Hulu Sungai Utara, and Enrengkang (Ariati et al. 2020).

Reservoirs are animals that contain parasites and act as a source of infection for humans. In the case of filariasis, some animals, such as cats (*Felis catus* Linnaeus, 1758), langurs (*Presbytis cristata*), long-tailed macaques (*Macaca fascicularis* (Raffles, 1821)), jungle cats (*Felis silvestris* Schreber, 1777), and dogs (*Canis familiaris*), can act as reservoir hosts for filarial nematodes and maintain the parasite's life cycle and transmission in endemic areas (Supriyono et al. 2017). Andiarsa et al. (2018) reported *B. malayi* in blood samples collected from dogs, cats, and long-tailed macaques based on a thin film examination in West Kotawaringin District, Central Kalimantan, Indonesia. *B. malayi* filaria was also detected in the blood samples of cats in Balangan District (Supriyono et al. 2017).

According to the regulation of the Ministry of Health of the Republic of Indonesia No. 94/2014 on filariasis management, Seluma District is categorized as a filariasis-endemic area in Bengkulu Province. This area has a microfilariae prevalence of more than 1% based on microfilariae surveys using the finger-prick blood survey method. A total of 22 chronic filariasis cases were reported in several sub-districts in Seluma District. Therefore, surveillance and detection activities for filariasis in vectors and reservoirs are necessary to determine the types of vectors, filaria, and reservoirs that play a role in filariasis transmission to prevent and control its transmission.

Surveillance and monitoring activities can begin by understanding the diversity and abundance of mosquitoes, filarial species, and reservoirs. Filariasis studies encompass a comprehensive understanding of humans as patients and sources of transmission, mosquitoes as vectors, animals as reservoir sources, and the environment as a supporting factor in interactions. By understanding mosquito behavior and habitat preferences, efforts to control and prevent the spread of the disease can be made more effective.

Based on the case data from Seluma District, it is important to determine the diversity and abundance of mosquitoes and filarial species and which animals act as reservoirs. This study analyzed research aims to analyze species diversity, mosquito behavior, and reservoir detection in filariasis-endemic areas in Bengkulu Province.

MATERIALS AND METHODS

Ethical approval

The authors obtained ethical approval to conduct this study from the Bengkulu Polytechnic of Ministry Health Ethics Committee Number. Kepk/395/08/2022.

Study area

This study was conducted from November 2022 to May 2023 in Seluma District, which comprises six villages: Pasar Tais (4°04′40″S, 102°34′25″E), Lubuk Lintang (4°03′36″S, 102°35′07″E), Talang Saling (4°04′24″S, 102°33′56″E), Penago 2 (4°11′45″S, 102°38′30″E), Mekar Sari (4°11′18″S, 102°36′07″E), and Tanah Abang (4°10′31″S, 102°36′16″E). Topographically, Seluma District is a hilly area with an altitude of 541 m above sea level and is geographically surrounded by plantations, swampy waters, and forests, which are suitable for life cycle and development of filariasis vector mosquitoes. These locations were specifically chosen due to their high filariasis endemicity, as evidenced by previous epidemiological data and reports from the Ministry of Health. The significant number of filariasis cases in this region made it a key area for investigating the dynamics of vector behavior and parasite transmission in endemic settings.

Procedures

Mosquito collection and identification

Mosquitoes were collected using two methods: Human Landing Collection (HLC) and Resting Collection (RC), conducted both indoors and outdoors. In the HLC method, two collectors were deployed one indoors and one outdoors. The RC method utilized an aspirator tube to gather mosquitoes resting on indoor surfaces (e.g., walls, furniture, dark areas) and outdoor resting places (e.g., foliage, shrubs, shaded areas). Collection occurred bi-monthly over a six-month period, from 18:00 to 06:00 WIB (UTC+07:00), with mosquitoes collected hourly for 50 minutes, followed by a 10-minute rest period. Three houses per village were purposively selected based on population characteristics. All mosquitoes were identified using the Walter Reed Biosystematics Unit (2023) identification keys for the genera *Aedes*, *Anopheles*, *Culex*, *Mansonia*, and *Armigeres*. Informed consent was obtained from all collectors prior to the start of the collection.

Capture and blood sampling of reservoir animals

The capture of reservoir animals in this study followed welfare protocols as per the government regulation of the Republic of Indonesia No. 95/2012 concerning veterinary public health and animal welfare. The captured animals included domestic animals comprising cats (*F. catus*) and dogs (*C. familiaris*) and wild animals (*M. fascicularis*). The sample size was calculated using the Levy and Lemeshow formula (Levy and Lemeshow 2008; Andiarsa et al. 2018). The total number of reservoir animal samples used in this study was 100, comprising 30 dogs, 40 cats, and 30 long-tailed macaques. Blood samples were collected once, with 0.5-1 mL of blood drawn at night from an accessible vein (cephalic vein, jugular vein, saphenous vein, or femoral vein) depending on the animal species. Each animal was tagged to facilitate sample identification.

Microfilariae detection in mosquitoes and reservoir animals

Detection of microfilariae in mosquitoes was performed using molecular methods. DNA extraction of microfilariae from mosquitoes and blood was performed using a Geneaid® Genomic DNA Mini Kit (Tissue) (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. Polymerase Chain Reaction (PCR) amplification was performed in 25-µL PCR mix volumes consisting of

12.5 µL of MyTaq™ Red mastermix (Bioline, United Kingdom), 7.5 µL of ddH2O, 1 µL of each primer, and 3 µL of DNA template. The thermal cycling program was as follows: 1 cycle of initial denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 30 s (denaturation), annealing at the annealing temperature of the primers for 30 s (Table 1), extension at 72°C for 7 min (extension), and final extension at 20°C for 2 min. PCR product analysis was performed via electrophoresis on a 1.2% agarose gel and visualized under an ultraviolet transilluminator. The primers, annealing temperatures, and gene lengths used in this study are presented in Table 1.

Detection of microfilariae in reservoir animals was performed using thin blood smears of 6 µL, which were then fixed with methanol and stained with Giemsa. Microfilariae were detected morphologically under a microscope at 40× magnification, based on size, presence of sheath, and head and tail morphologies (Dietrich et al. 2019; Mathison et al. 2019).

Data analysis

Mosquito species diversity was calculated on the basis of the dominance index (D), Simpson diversity index (1-D), Shannon diversity index (H), and Shannon - Weiner evenness (E) using PAST 4.0 (Magurran 2013). The results of the species diversity index values were grouped into three categories: H'<1 = low species diversity level, 1<H'<3 = moderate species diversity level, and H'>3 = high species diversity level (Roswell 2021). Mosquito density was Measured using man Hour Density (MHD) and Man Biting Rate (MBR) values based on the number of mosquitoes collected using the HLC method over 12 h.

RESULTS AND DISCUSSION

Diversity and abundance of mosquitoes

In total, 1,346 individual mosquitoes were collected indoors and outdoors using HLC and RC, and 17 species were identified (Table 2). In general, the percentage of mosquitoes collected outdoors using HLC and RC was higher than that collected indoors. The highest relative abundance of species was observed in *Culex pseudovishnui* Colless, 1957 (29.3%), followed by *Culex vishnui* Theobald, 1901 and *Culex quinquefasciatus* Say, 1823 with relative abundance values of 24.5 and 23.5%, respectively.

Table 1. Primers, annealing temperatures, and gene lengths used in filaria detection

Primer	Sequence (5′→3′)	Annealing temperature (°C)	Size (bp)	Reference
ITS1F	GGTGAACCTGCGGAAGGATC	57.8	482	Medeiros et al. 2018
ITS1R	GCGAATTGCAGACGCATTGAG			
HhaI F	GCGCATAAATTCATCAGC	51	322	Albers et al. 2014
HHaI R	GCGCAAAACTTAATTACAAAAGC			
WBF	CACCGGTATCGAGATTAATT	55	780	Abdel-Shafi et al. 2017
WBR	TTGTTCCTCTATTTGAGACC			
WBF	CACCGGTATCGAGATTAATT	60	400	
WB2	TGGATGTATGTCAAAAAGCA			

Table 2. Mosquito species collected using human landing and resting collections methods in endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023

Species	Number of species (%)	Human landing collection		Resting collection	
		Indoor (%)	Outdoor (%)	Indoor (%)	Outdoor (%)
Culex quinquefasciatus Say, 1823	316 (23.5)	56 (23.2)	123 (29.3)	31 (16.7)	106 (21.2)
Culex hutchinsoni Barraud, 1924	90 (6.7)	17 (7.1)	19 (4.5)	22 (11.8)	32 (6.4)
Culex gelidus Theobald, 1901	35 (2.6)	10 (4.1)	8 (1.9)	3 (1.6)	14 (2.8)
Culex pseudovishnui Colless, 1957	394 (29.3)	94 (39.0)	98 (23.3)	65 (34.9)	137 (27.5)
Culex vishnui Theobald, 1901	330 (24.5)	47 (19.5)	109 (26.0)	49 (26.3)	125 (25.1)
Armigeres subalbatus (Coquillett, 1898)	101 (7.5)	6 (2.5)	43 (10.2)	10 (5.4)	42 (8.4)
Aedes aegypti (Linnaeus, 1762)	18 (1.3)	1 (0.4)	5 (1.2)	1 (0.5)	11 (2.2)
Aedes albopictus (Skuse, 1894)	4 (0.3)	0 (0)	2 (0.5)	0 (0)	2 (0.4)
Anopheles tessellatus Theobald, 1901	11 (0.8)	7 (2.9)	1 (0.2)	0 (0)	3 (0.6)
Anopheles vagus Döntiz, 1902	6 (0.4)	1 (0.4)	0 (0)	1 (0.5)	4 (0.8)
Anopheles minimus Theobald, 1901	10 (0.7)	0 (0)	2 (0.5)	0 (0)	8 (1.6)
Anopheles indefinitus (Ludlow, 1904)	12 (0.9)	1 (0.4)	3 (0.7)	1 (0.5)	7 (1.4)
Anopheles sundaicus (Rodenwaldt, 1948)	2 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.4)
Anopheles barbirostris Wulp, 1884	3 (0.2)	0 (0)	2 (0.5)	0 (0)	1 (0.2)
Anopheles maculatus Theobald, 1901	2 (0.1)	1 (0.4)	0 (0)	0 (0)	1 (0.2)
Anopheles sp.	1 (0.1)	0 (0)	1 (0.2)	0 (0)	0 (0)
Mansonia annulata Leicester, 1908	11 (0.8)	0 (0)	4 (1.0)	3 (1.6)	4 (0.8)
Total	1346 (100)	241 (17.9)	420 (31.2)	186 (13.8)	499 (37.1)

The highest number of mosquito species captured using the HLC method outdoors was 14 species. The diversity of mosquito species captured using both methods, both outdoors and indoors, was classified into the moderate species diversity level category based on the Shannon index value, and no mosquito species dominated according to the dominance index value (Table 3).

Density and biting behavior of mosquitoes

The mosquito species with the highest density outdoors was *C. quinquefasciatus* at 10.3 mosquitoes/person/night, whereas *C. pseudovishnui* had the highest density indoors at 7.8 mosquitoes/person/night (Table 4).

The mosquitoes in the study area exhibited different landing activities both indoors and outdoors (Figure 1). Indoors, peak activity of *C. pseudovishnui* occurs between 18:00 and 19:00, followed by a decrease in biting activity until midnight and with a subsequent increase between 02:00 and 06:00. Outdoors, *C. quinquefasciatus* exhibits two peaks in biting activity: between 19:00-20:00 and 22:00-23:00, with the highest peak occurring during the first peak.

Detection of microfilariae in mosquitoes

The results of filaria detection in mosquito samples using molecular methods are presented in Table 5. Microfilariae were not detected in any of the mosquito samples using PCR methods with the ITS-1, HHa1, and Wb primers.

Table 4. Man biting rate of mosquito species in filariasis-endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023

Species	MBR (Mosquito/person/night)	
	Indoor	Outdoor
Culex quinquefasciatus	4.7	10.3
Culex hutchinsoni	1.4	1.6
Culex gelidus	0.8	0.7
Culex pseudovishnui	7.8	8.2
Culex vishnui	3.9	9.1
Armigeres subalbatus	0.5	3.6
Aedes aegypti	0.1	0.4
Aedes albopictus	0.0	0.2
Anopheles tessellatus	0.6	0.1
Anopheles vagus	0.1	0.0
Anopheles minimus	0.0	0.2
Anopheles indefinitus	0.1	0.3
Anopheles sundaicus	0.0	0.0
Anopheles barbirostris	0.0	0.2
Anopheles maculatus	0.1	0.0
Anopheles sp.	0.0	0.1
Mansonia annulata	0.0	0.3

Table 3. Abundance, richness, diversity, evenness, and dominance of mosquitoes in filariasis-endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023

Index	Collection method			
	Indoor HLC	Outdoor HLC	Indoor HLC	Outdoor HLC
Number of species (S)	11	14	10	16
Number of individuals (N)	241	420	186	499
Dominance (D)	0.252	0.221	0.237	0.196
Simpson (1-D)	0.748	0.779	0.763	0.804
Shannon (H)	1.630	1.736	1.645	1.903
Shannon −Weiner Evenness (E)	0.464	0.405	0.518	0.419

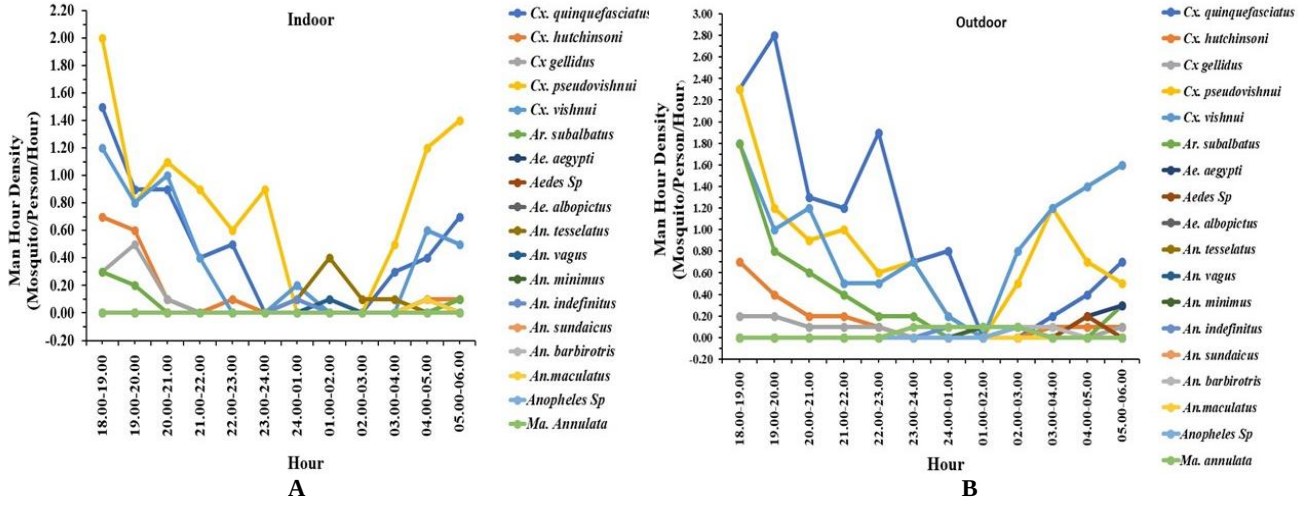

Figure 1. Man hour density outdoor (A) and indoor (B) by mosquito species in filariasis-endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023. Note: *Cx.*: *Culex*; *Ar.*: *Armigeres*; *Ae.*: *Aedes*; *An.*: *Anopheles*; *Ma.*: *Mansonia*

Table 5. Results of polymerase chain reaction based on mosquito species in filariasis-endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023

Species of mosquito	Number of mosquitoes	Number of pools	Primers		
			ITS-1	HhaI	Wb
Culex quinquefasciatus Say, 1823	316	14	-	-	-
Culex hutchinsoni Barraud, 1924	90	5	-	-	-
Culex gelidus Theobald, 1901	35	5	-	-	-
Culex pseudovishnui Colless, 1957	394	14	-	-	-
Culex vishnui Theobald, 1901	330	12	-	-	-
Armigeres subalbatus (Coquillett, 1898)	101	7	-	-	-
Aedes aegypti (Linnaeus, 1762)	18	1	-	-	-
Aedes albopictus (Skuse, 1894)	2	1	-	-	-
Anopheles tessellatus Theobald, 1901	11	1	-	-	-
Anopheles vagus Döntiz, 1902	6	3	-	-	-
Anopheles minimus Theobald, 1901	10	1	-	-	-
Anopheles indefinitus (Ludlow, 1904)	12	1	-	-	-
Anopheles sundaicus (Rodenwaldt, 1948)	2	1	-	-	-
Anopheles barbirostris Wulp, 1884	3	1	-	-	-
Anopheles maculatus Theobald, 1901	2	1	-	-	-
Anopheles sp.	1	1	-	-	-
Mansonia annulata Leicester, 1908	11	1	-	-	-

Note: -: PCR results showed negative

Table 6. Microscopic analysis of the presence of microfilariae in reservoirs in filariasis-endemic areas in Bengkulu Province, Indonesia from November 2022 to May 2023

Species of reservoir	Number of reservoirs (%)	Microfilariae test results	
		Positive (%)	Negative (%)
Canis familiaris	30 (30%)	4 (13.3%)	26 (86.6%)
Felis catus Linnaeus, 1758	40 (40%)	0 (0.0%)	40 (100%)
Macaca fascicularis (Raffles, 1821)	30 (30%)	0 (0.0%)	30 (100%)

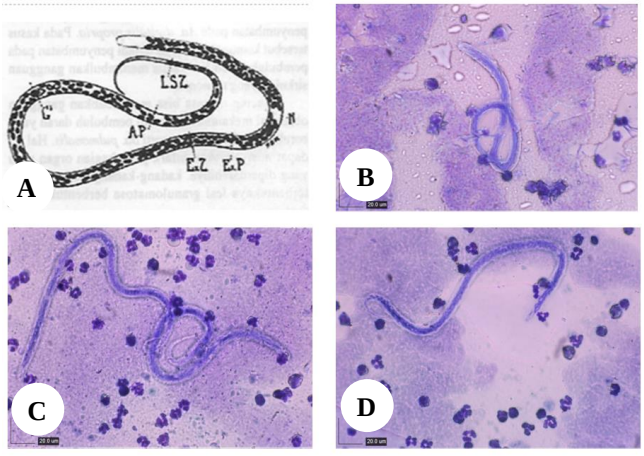

Figure 2. Morphology of *Dirofilaria immitis* (Leidy, 1856) Railliet & Henry, 1911 based on reference (A) and blood slides of *D. immitis* in dogs in filariasis-endemic areas of Bengkulu Province (B - D). Figure (A) was taken from Soulsby EJL (1968)

Microfilariae detection in reservoirs

Microscopic analysis of the blood samples from the reservoir animals showed that 100% of the captured cats and long-tailed macaques had no filarial nematode larvae, whereas 13.3% (4 dogs) of the total number of dogs captured were positive for filarial worms (Table 6). Morphological identification revealed microfilariae suspected to be *Dirofilaria immitis* (Leidy, 1856) Railliet & Henry, 1911 microfilariae (Figure 2).

Discussion

The collection of mosquitoes in filariasis-endemic areas aims to identify the mosquito species circulating in these areas and assess their potential as filariasis vectors. The HLC method is used to determine which mosquito species prefer to bite humans. The methods used in this study serve as indicators of vector human interactions and provide a clearer picture of where (indoors or outdoors) and when (night time) exposure to filariasis vectors occurs (Monroe et al. 2022). *Culex pseudovishnui* and *C. quinquefasciatus* were the most common mosquito species detected indoors and outdoors. To date, there are no reports of *C. pseudovishnui* as a vector of filariasis. This species is commonly reported as a vector of the Japanese encephalitis virus (Saiwichai et al. 2023). *Culex quinquefasciatus* is an important vector of several pathogens, including the nematode *W. bancrofti* and West Nile virus, in tropical and subtropical countries. This species has been reported as a major vector of filariasis in Bekasi, Tangerang, Pekalongan, and Lebak in Java (Lee and Ryu 2019).

Mosquito density, as measured using MBR and MHD, showed significant variations, with bites from all mosquito species occurring throughout the night, both indoors and outdoors. The higher the density of a mosquito species, the greater its potential as a disease vector. According to Table

2, nine mosquito species bit both indoors and outdoors, five species bit only bit outdoors, and two species bit only indoors. An interesting finding of this study is that *Anopheles vagus* Döntiz, 1902 and *Anopheles maculatus* Theobald, 1901 species prefer to bite indoors in filariasis-endemic areas in Bengkulu province. Al-Amin et al. (2023) reported similar findings, stating that *A. vagus* is anthropophilic and tends to rest indoors in Bandarban, Bangladesh. This is in contrast with previous reports indicating that *A. vagus* is zoophilic, exophilic, and exophagic (Dalilah et al. 2024).

In addition, Walter Reed Biosystematics Unit (2023) reported that *A. maculatus* is highly anthropophilic. These findings suggest variations in mosquito behavior based on geographical location and environmental conditions, which are important considerations for in vector control strategies. Further research is required to explore the factors influencing these behavioral changes and their impact on disease transmission in endemic areas.

Wuchereria bancrofti DNA was not detected in all mosquito sample pool using PCR. The Ministry of Health of the Republic of Indonesia (2014) stated that filariasis in Bengkulu Province is caused by *B. malayi*, which is transmitted by *Mansonia uniformis* (Theobald, 1901), *Mansonia annulate* Leicester, 1908, *Mansonia dives* (Schiner, 1868) and *Mansonia bonneae* Edwards, 1930. Suzuki et al. (1981) also reported that the vectors of *B. malayi* filariasis in Bengkulu Province are *Mansonia* mosquitoes and *Anopheles nigerrimus* Giles, 1900. In this study, *M. Annulata was* found: however, no *A. nigerrimus* detected. Although *A. nigerrimus* was not found in the study area, eight other species of *Anopheles* were found: *Anopheles tessellatus* Theobald, 1901, *A. vagus*, *Anopheles minimus* Theobald, 1901, *Anopheles indefinitus* (Ludlow, 1904), *Anopheles sundaicus* (Rodenwaldt, 1948), *Anopheles barbirostris* Wulp, 1884 and *A. maculatus*. *A. vagus* is a vector of *B. malayi* filariasis in West Nusa Tenggara (Lee et al. 1983; Willa 2011; Ariati et al. 2020). No mosquitoes infected with filariasis were detected; however, this does not definitively indicate the absence of filariasis transmission in our study site. In contrast, this may be due to the small number of mosquitoes in each collection and the small number of infected mosquitoes. This is a significant challenge in the surveillance of filariasis transmission, because a large number of mosquito traps is required when transmission is low in the study area.

Microfilarial examination of *W. bancrofti* and *B. malayi* in the reservoir animals in the study area did not reveal any filarial presence. However, *D. immitis* larvae were found in the dog blood samples. The species *D. immitis* causes zoonoses in humans, mainly affecting the lungs. Human infections with *D. immitis* have been reported in many countries, including the United States, South America, Europe, Japan, Australia (Simón et al. 2012), Italy, and Brazil (Perles et al. 2024). *Dirofilaria immitis* is a cosmopolitan species that is transmitted by different mosquito species, including *Aedes* spp, *Anopheles* spp, *Culex* spp, and *Ochlerotatus* spp. (Silaghi et al. 2017; Riahi et al. 2021).

In conclusion, we successfully identified 17 different mosquito species captured both indoors and outdoors using HLC and RC. The diversity of mosquito species in the study area was categorized as moderate. Among the captured species, *C. pseudovishnui* exhibited the highest relative abundance, followed by *Culex vishnui* and *C. quinquefasciatus*. The biting behavior of mosquitoes significantly varied between indoor and outdoor environments. Specifically, *C. pseudovishnui* exhibited the highest density indoors, whereas *C. quinquefasciatus* was the most dominant species outdoors. Despite comprehensive sampling and analysis, we detected no microfilaria DNA in any of the mosquito samples from the study area based on PCR examination. These findings indicate that, within the scope of this study, there is no evidence of microfilaria presence in local mosquito populations. However, the detection of *D. immitis* in the dog reservoir animal samples indicates that there remains a risk of filarial infections being maintained and potentially transmitted in the study area through other vectors or mechanisms. These findings highlight the importance of continuous surveillance and targeted vector control strategies to reduce the risk of filariasis and other mosquito-borne diseases. Further research is required to explore the ecological dynamics of mosquito populations and their interactions with human and animal hosts, to develop more effective disease prevention and control interventions.

ACKNOWLEDGEMENTS

We would like to thank the Government of Seluma District, Bengkulu Province, for providing facilities and infrastructure during this study, the volunteers who were willing to collect mosquitoes, and the team members who helped collect mosquitoes in the field. We are grateful to the staff of the Laboratory of Medical Entomology, School of Veterinary Medicine and Biomedical Sciences, Institut Pertanian Bogor, Indonesia, for allowing us to conduct PCR tests on mosquito samples, and the Head of the Regional Health Laboratory, Bengkulu Province, Indonesia, for allowing us to collect blood samples from animals.

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