SEHATRAKYAT (Jurnal Kesehatan Masyarakat)
https://journal.yp3a.org/index.php/sehatrakyat
DOI: 10.54259/sehatrakyat.v5i3.7488
e-ISSN 0852-1239 | p-ISSN 2829-9299
Vol. 5 No. 3 (Agustus 2026) 1226-1236
Diterima Redaksi: 17-04-2026 | Selesai Revisi: 11-06-2026 | Diterbitkan Online: 07-08-2026

Dual Periodicity and Molecular Evidence of Brugia Malayi Transmission Dynamics in Banyuasin District, South Sumatra, Indonesia

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Abstract

Lymphatic filariasis remains one of the most neglected tropical diseases, characterized by complex transmission dynamics involving parasites, vectors, hosts, and environmental determinants. Understanding the periodicity of Brugia malayi and the molecular confirmation of potential vectors are crucial for optimizing control and elimination strategies in endemic regions. Objective: This study aimed to characterize the transmission dynamics of Brugia malayi by analyzing its microfilarial periodicity and confirming vector species through molecular identification. Methods: A cross-sectional field study was conducted from April 2017 to May 2018 in Banyuasin District, South Sumatra. Peripheral blood samples from humans and domestic cats, as well as adult mosquitoes, were collected and examined microscopically for microfilariae. Polymerase chain reaction (PCR) targeting the yACA gene of Brugia spp. was used for molecular confirmation. Microfilarial periodicity was assessed through hourly finger-prick sampling over 24 hours and analyzed using the Aikat and Das formula. Results: Two of six human subjects in Sedang Village were positive for Brugia malayi microfilariae with an average density of 150.3 mf/mL, while one cat from Muara Sugih was positive for Dirofilaria repens. PCR detected Brugia malayi DNA in two Mansonia spp. samples (I4 and I8) with 93–95% identity to reference strain MG030487.1. The periodicity exhibited a dual pattern diurnal (peak at 15:00) and subperiodic nocturnal distinct from the typical nocturnal rhythm reported elsewhere in Indonesia. Conclusion: The detection of dual periodicity and molecular confirmation of Brugia malayi in Mansonia spp. highlight potential adaptive transmission patterns influenced by local ecological conditions in Banyuasin's wetland ecosystems.

Keywords: *Brugia Malayi*, Filariasis, Periodicity, *PCR*, *Mansonia Spp*.

INTRODUCTION

Lymphatic filariasis, caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, remains a major public health problem in tropical and subtropical regions, including Indonesia. Although the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has implemented mass drug administration (MDA) for over two decades, transmission persists in several endemic provinces such as South Sumatra, Kalimantan, and Papua,

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indicating the complexity of local transmission dynamics. The persistence of this disease reflects multifactorial interactions among parasites, vectors, hosts, and environmental factors that sustain ongoing transmission cycles.

Vector surveillance plays a crucial role in understanding transmission persistence after MDA. Entomological monitoring enables the detection of residual infections in mosquito populations and helps evaluate the effectiveness of elimination programs. Molecular identification of filarial DNA in mosquitoes provides a sensitive tool for detecting ongoing transmission even when human microfilaremia prevalence becomes very low. A recent study in Bekasi District, West Java, demonstrated that even after several rounds of MDA, *Brugia malayi* DNA was still detected in vector mosquitoes, suggesting ongoing low-level transmission and the need for sustained surveillance (Astuti et al., 2023; Galgamuwa LS et al., 2025; Wayangkau EC et al., 2025).

The microfilarial periodicity of *Brugia malayi* plays a crucial role in determining mosquito infectivity and transmission efficiency. Periodicity refers to the rhythmic fluctuation of microfilariae in peripheral blood that aligns with the biting activity of vector mosquitoes, optimizing parasite uptake and transmission success. Previous studies in Indonesia have shown a predominance of nocturnal periodicity of *Brugia malayi* in human infections (Edyansyah et al., 2023; Rajamanickam et al., 2025). However, recent research from Kalimantan and coastal regions revealed the presence of diurnal and subperiodic types of microfilarial periodicity, suggesting ecological or behavioral adaptation of parasite–vector populations (Rahayu et al., 2023). These variations may influence local transmission dynamics and affect the effectiveness of surveillance and control strategies.

Banyuasin District in South Sumatra represents a unique ecological landscape characterized by wetlands, agricultural zones, and mangrove ecosystems that provide ideal breeding habitats for *Mansonia* mosquitoes the principal vectors of *Brugia malayi*. Despite continuous endemicity, studies investigating the periodicity of microfilariae and molecular confirmation of local mosquito vectors remain limited in this region. Comprehensive studies integrating parasitological and entomological approaches are therefore needed to better understand the persistence of transmission in Banyuasin.

Therefore, this study aimed to characterize the transmission dynamics of *Brugia malayi* in Banyuasin District, South Sumatra, through an integrated analysis of microfilarial periodicity and molecular identification of mosquito vectors. The findings are expected to provide new insights into local ecological adaptation, host–vector relationships, and implications for the optimization of filariasis control and elimination strategies in Indonesia. The findings are expected to provide new insights into local ecological adaptation, host–vector relationships, and the persistence of transmission following MDA implementation, as well as to support optimization of filariasis surveillance, control, and elimination strategies in Indonesia.

METHOD

A cross-sectional observational study was conducted from April 2017 to May 2018 in two lymphatic filariasis–endemic villages: Sedang and Muara Sugih, located in Banyuasin District, South Sumatra Province, Indonesia. The study area is characterized by wetland ecosystems, agricultural fields, and residential settlements that provide suitable habitats for Mansonia mosquitoes, the known vectors of *Brugia malayi*.

Mosquito sampling was performed using two established entomological techniques: the human landing catch (HLC) method. Collected mosquitoes were identified morphologically based on genus and species keys using established entomological references. The head–thorax section of each mosquito was dissected microscopically to detect the presence of filarial larval stages. Peripheral blood samples were collected from

six consenting adult human participants and nineteen domestic cats residing in the same households. Human inclusion criteria included individuals aged ≥18 years who had lived in the study area for at least six months prior to sampling and were willing to participate by providing informed consent. Individuals who had recently migrated to the area, had incomplete demographic information, or declined participation were excluded from the study. For animal sampling, only local domestic cats that had resided in the same household environment were included to ensure representation of long-term environmental exposure within the endemic area.

Thick and thin blood smears from human participants were prepared and stained with Giemsa, then examined under oil immersion (1000× magnification). The presence and density of Brugia malayi microfilariae were recorded as the number of microfilariae per milliliter (mf/mL) of blood. To assess microfilarial periodicity, finger-prick blood samples were collected hourly for a 24-hour period from individuals found positive during screening.

Polymerase chain reaction (PCR) analysis was performed at the Eijkman Institute for Molecular Biology, Jakarta, using Filaria-Forward (5′-GCGCATAAATTCATCAGC-3′) and Filaria-Reverse (5′-GCGCAAAACTTAATTACAAAAGC-3′) primers targeting the yACA gene of Brugia spp. The expected amplicon sizes were 326 bp for B. malayi and B. timori, and 338 bp for B. pahangi. Amplified products were visualized by electrophoresis on 2% agarose gel stained with ethidium bromide and compared against known reference strains for confirmation.

Microfilarial periodicity was analyzed using the Aikat and Das formula to determine the mean density (m), amplitude (A), and phase angle (K) of hourly microfilarial counts. The periodicity index (D) was calculated to classify the rhythm type: values greater than 50 were categorized as periodic, whereas values below 50 indicated subperiodic patterns. Statistical analyses were performed using descriptive and comparative methods to evaluate diurnal variation and periodic rhythmicity. All data were entered and analyzed using IBM SPSS Statistics version 25.0 and Microsoft Excel for data tabulation, periodicity calculations, and graphical visualization of hourly microfilarial density patterns.

Ethical approval was obtained from the Health Research Ethics Committee of RSUP Dr. Mohammad Hoesin–Faculty of Medicine, Universitas Sriwijaya (Approval No: 07/KEPK-RSMH/2017).

RESULTS

Microscopic examination of peripheral blood samples revealed that two out of six person participants were positive for *Brugia malayi* microfilariae, with an average microfilarial density of 150.3 mf/mL of blood. Among domestic animal samples, one of six cats examined in Muara Sugih Village was found to harbor *Dirofilaria repens*, characterized by an average microfilarial density of 484.3 mf/mL and the presence of two terminal nuclei at the posterior end. No third stage (L3) larvae were detected microscopically in any mosquito specimens collected from April 2017 to May 2018, indicating the absence of active larval development at the time of sampling.

Molecular analysis using polymerase chain reaction (PCR) targeting the yACA gene confirmed the presence of *Brugia malayi* DNA in two mosquito samples (designated I4 and I8). Sequence alignment showed a 93–95% homology with the GenBank reference strain *Brugia malayi* (Accession No. MG030487.1), confirming active vector–parasite circulation in Sedang Village during the study period. This finding supports the microscopic evidence of ongoing transmission in the local population.

Hourly examination of peripheral blood over a 24-hour cycle demonstrated detectable microfilariae between 09:00 and 24:00 hours, with prominent density peaks observed at 15:00 and 24:00. One participant exhibited a diurnal periodic pattern with statistical parameters F = 7.82 and D = 82.4, while another demonstrated a subperiodic nocturnal pattern (F = 2.714; D = 19.8). These results indicate the coexistence of both diurnal and subperiodic nocturnal microfilarial rhythms in the Banyuasin population, suggesting possible ecological or vector-related adaptation influencing parasite periodic behavior.

Figure 1. Microfilarial of Brugia malayi (10x100).

Figure 2. Microfilarial of Dirofilaria repens (10x100)

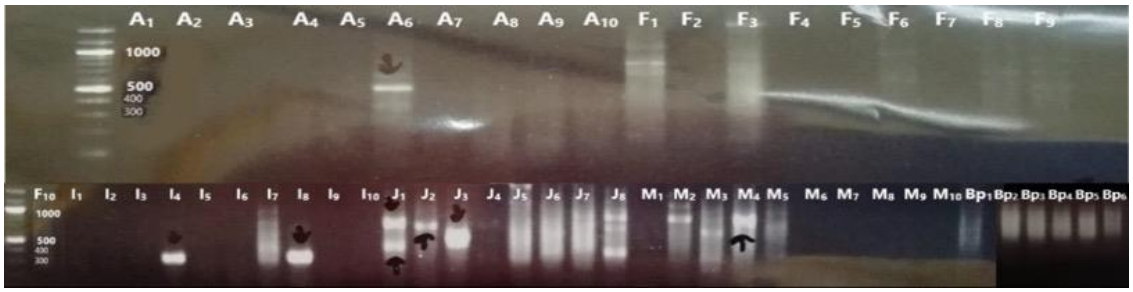

Figure 3. PCR confirmed the presence of Brugia malayi DNA

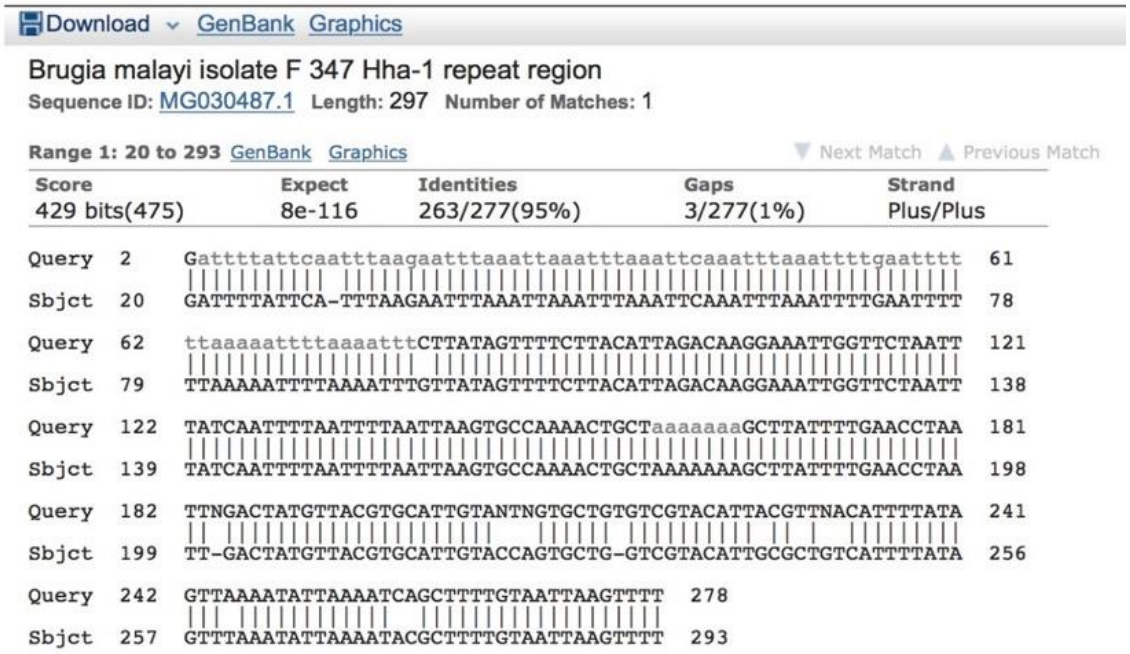

Figure 4. Sequencing of Brugia malayi DNA

Table 1. Time of examination of peripheral blood

Time	Density of Microfilarial					
	P1	P2	P3	P4	P5	P6
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	1	0	0	0	0	0
10	1	0	0	0	0	0
11	1	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	1	0	0	0	0	0
15	3	0	0	0	0	0
16	1	0	0	0	0	0
17	1	0	0	0	0	0
18	1	0	0	0	0	0
19	1	1	0	0	0	0
20	0	0	0	0	0	0
21	1	0	0	0	0	0
22	1	0	0	0	0	0
23	1	0	0	0	0	0
24	2	1	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
Total	16	2	0	0	0	0

Table 2. Analysis of Microfilarial periodicity

Statistic Analysis	P1 1	P2	P3	P4	P5	P6
Y	16	2	0	0	0	0
Y2	24	02	0	0	0	0
Y cos 15h	-1,487	1,259	0	0	0	0
Y sin 15 h	-6,419	-0,966	0	0	0	0
M	0,667	0,667	0	0	0	0
B	-0.124	0,105	0	0	0	0
C	-0,535	-0,0805	0	0	0	0
A	0,549	0,132	0	0	0	0
K	16.06’30”	00.07;08”	0	0	0	0
F	7,82	2,714	0	0	0	0
D	82,4	19,8	0	0	0	0

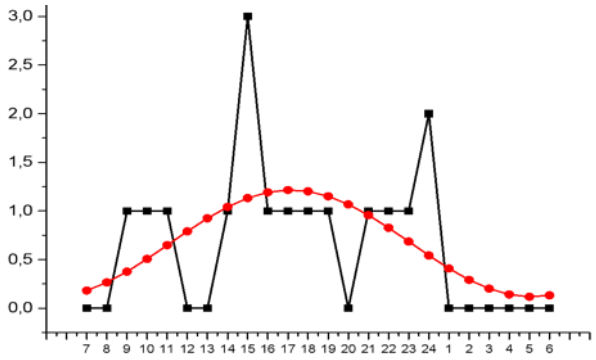

Figure 5. Diurnal Periodic Circadian Rhythm of Brugia malayi (P1).

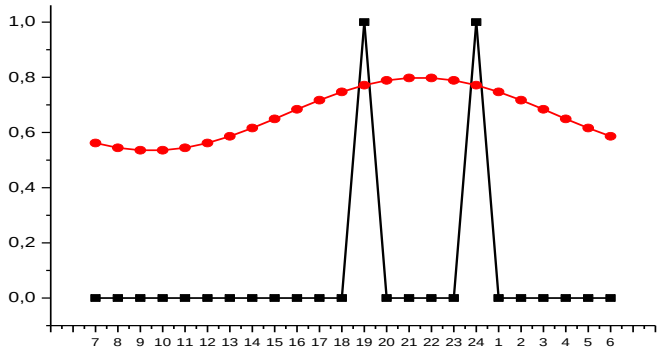

Figure 6. Nocturnal Subperiodic Non-Circadian Rhythm of Brugia malayi (P2).

The red line indicates the expected threshold/standard pattern representing microfilarial periodicity, while the blue line represents the presence and fluctuation of microfilariae observed in subjects P1 and P2.

Table 3. Species Composition of *Mansonia spp.*

Species	**Sedang n (%)**	**Muara Sugih n (%)**	**Total n (%)**
M. annulifera	2,226 (53.3)	659 (21.5)	2,885 (39.9)
M. uniformis	756 (18.1)	1,597 (52.1)	2,353 (32.5)
M. indiana	1,194 (28.6)	807 (26.4)	2,001 (27.6)
Total	**4,176**	**3,063**	**7,239**

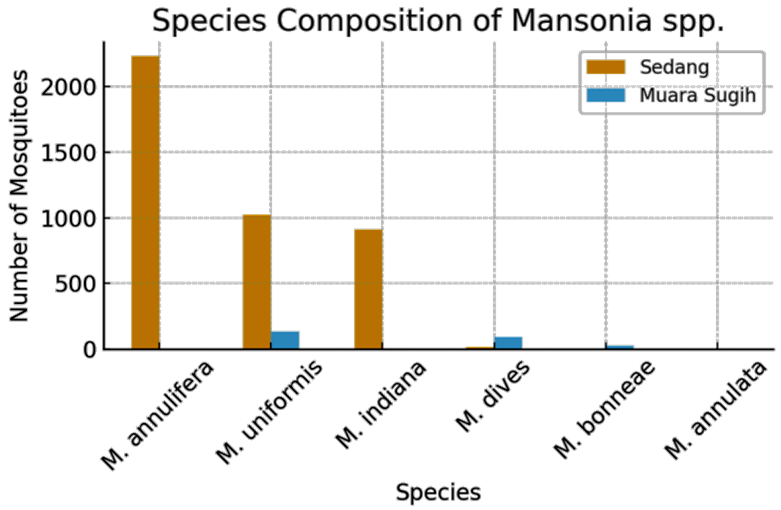

Figure 7. Species Composition of *Mansonia spp.*

DISCUSSION

This study provides the first molecular and chronobiological evidence of dual periodicity of *Brugia malayi* in South Sumatra, Indonesia. The coexistence of diurnal and subperiodic nocturnal forms within the same locality suggests a dynamic adaptation of *Brugia malayi* to the ecological conditions and host–vector interactions in Banyuasin's wetland ecosystem. These findings differ from earlier reports across Indonesia that described *Brugia malayi* as predominantly nocturnal, with microfilarial peaks occurring between 22:00–02:00 hours (Edyansyah et al., 2023). The diurnal peak observed at 15:00 in the present study may reflect parasite synchronization with altered human–vector contact patterns and ecological changes affecting *Mansonia* activity cycles. This altered periodicity pattern has important implications for public health interventions and vector control strategies. Current community prevention programs are predominantly focused on nighttime protection measures, such as the use of bed nets and avoidance of nocturnal mosquito exposure. However, the presence of daytime or subperiodic transmission patterns suggests that exposure to infective mosquito bites may also occur during daily outdoor activities, including farming, fishing, and agricultural work commonly performed during daylight hours in Banyuasin. Consequently, reliance solely on nighttime interventions may be insufficient to interrupt transmission in this setting. Public health strategies should therefore expand to include daytime protective measures such as topical repellents, protective clothing, environmental management of aquatic vegetation, and community education regarding daytime vector exposure. In addition, entomological surveillance should incorporate both diurnal and nocturnal mosquito collection schedules to better capture local transmission dynamics.

A similar shift in filarial periodicity has been reported in coastal and riverine ecosystems of Kalimantan, where *Brugia malayi* exhibited subperiodic and diurnal tendencies associated with *Mansonia annulifera* and *Mansonia uniformis* activity during daylight hours (Rahayu et al., 2023). These chronobiological adaptations likely arise from vector feeding behavior plasticity, temperature and humidity variations, and anthropogenic influences such as land conversion, deforestation, and agricultural irrigation (Suh et al.,2020; Chaves et al.,2024; Zahouli et al.,2017). The persistence of both rhythmic types in Banyuasin may thus represent a transitional adaptation between traditional nocturnal transmission cycles and newly emerging diurnal foci driven by environmental pressures.

The molecular detection of *Brugia malayi* DNA in *Mansonia* mosquitoes provides compelling evidence of active transmission post-MDA (Mass Drug Administration). Similar post-MDA persistence of filarial DNA has been demonstrated in Bekasi District, West Java, where *Brugia* sequences were still identified in vector populations years after repeated MDA rounds (Astuti et al., 2023). This indicates that vector infection monitoring using PCR based tools can reveal ongoing low-level transmission that is undetectable through routine microscopy or antigen tests (Ihsan et al., 2024). Thus, integrating molecular surveillance into post-MDA programs is critical for validating elimination success.

The concurrent detection of *Dirofilaria repens* in domestic cats further enriches the ecological narrative of vector–parasite interactions in Banyuasin. The presence of this zoonotic filarial species implies potential spillover risks, underscoring the importance of One Health surveillance that encompasses human, animal, and vector populations. The detection of Dirofilaria repens in cats living near human households suggests that domestic animals may serve as reservoir hosts that contribute to sustained zoonotic transmission within the community. In endemic wetland settlements such as Banyuasin, where humans, domestic animals, and mosquito vectors share overlapping ecological spaces, the risk of accidental human infection may increase through repeated mosquito exposure. Although human dirofilariasis is frequently underdiagnosed in tropical regions, the coexistence of infected animal reservoirs and competent mosquito vectors creates favorable conditions for spillover transmission. These findings highlight the importance of incorporating veterinary surveillance and animal reservoir monitoring into filariasis control programs, particularly in areas with persistent vector abundance and ongoing transmission.Previous studies in Sri Lanka and India have documented similar *Dirofilaria repens* reservoirs among domestic pets in filariasis-endemic regions, suggesting that domestic animals can act as reservoirs for cross-species transmission, thereby complicating elimination efforts (Thilakarathne SS et al.,2023; Atapattu U et al.,2023; Remya M et al., 2025).

The identification of both *Brugia malayi* and *Dirofilaria repens* in sympatric ecosystems highlights the need for integrated ecological modeling of vector–host–parasite networks. Wetland ecosystems such as Banyuasin serve as breeding grounds for *Mansonia spp.*, whose larval stages depend on aquatic vegetation. Habitat changes—such as increased vegetation density and agricultural irrigation—could enhance vector proliferation and modify biting activity patterns, facilitating cross-transmission between humans and animals (Nugroho et al., 2023; Diekmann I et al., 2025).

Overall, the findings from this study suggest that *Brugia malayi* transmission in South Sumatra is undergoing localized ecological adaptation characterized by altered periodicity, molecularly confirmed persistence post-MDA, and coexistence with zoonotic filariae. This pattern calls for region-specific vector control strategies that integrate entomological, molecular, and ecological data. Control programs must expand beyond chemotherapeutic approaches to include targeted vector habitat management and community-level One Health interventions addressing both human and animal reservoirs.

Limitations

The main limitation of this study is the small sample size and the absence of longitudinal entomological data across seasons. Future research should incorporate spatiotemporal surveillance using GIS-based ecological modeling, larger sample populations, and genetic analysis of vector feeding preferences to elucidate the adaptive mechanisms shaping filarial periodicity.

Table 4. Key Comparative Insights

Aspect	Present Study (Banyuasin, South Sumatra)	Comparable Studies
Periodicity	Dual (diurnal + subperiodic nocturnal)	Nocturnal (Edyansyah et al., 2023); Subperiodic diurnal (Rahayu et al., 2023)
Vector confirmed	Mansonia spp. (PCR positive)	Mansonia uniformis, M. annulifera (Astuti et al., 2023)
Post-MDA detection	Yes, ongoing B. malayi DNA	Similar persistence in Bekasi (Astuti et al., 2023)
Zoonotic coinfection	Dirofilaria repens in cats	Zoonotic D. repens in Sri Lanka and India (Subramanian et al., 2022)

In summary, this study contributes new evidence that *Brugia malayi* in Banyuasin exhibits dual periodicity and molecularly verified transmission via Mansonia mosquitoes despite prior MDA campaigns. The findings underscore the evolving nature of filarial ecology and advocate for adaptive, data-driven surveillance frameworks tailored to Indonesia's diverse ecological settings.

CONCLUSION

This study provides the first chronobiological and molecular evidence of dual periodicity of *Brugia malayi* in Banyuasin District, South Sumatra. The coexistence of diurnal and subperiodic nocturnal forms indicates adaptive transmission persistence driven by local ecological and behavioral factors. Molecular detection of Brugia *malayi* DNA in *Mansonia* mosquitoes confirms ongoing transmission despite mass drug administration (MDA), suggesting that parasite–vector synchronization continues to sustain transmission within wetland environments.

The findings highlight the need to strengthen integrated surveillance systems that combine molecular diagnostics, vector monitoring, and environmental assessment. Current MDA-based approaches should be complemented by targeted vector control, focusing on Mansonia breeding habitats associated with aquatic vegetation and agricultural irrigation. Additionally, the detection of zoonotic *Dirofilaria repens* in domestic cats reinforces the importance of a One Health approach, linking human, animal, and environmental health to address potential cross-species transmission risks. Public health policies in endemic regions must therefore prioritize community education, animal reservoir screening, and eco-based vector management.

Future studies should employ longitudinal and spatiotemporal analyses to examine seasonal variation in microfilarial periodicity, vector biting behavior, and parasite–vector interactions. The use of geospatial modeling, molecular phylogenetics, and climate data integration will provide deeper insight into environmental drivers influencing adaptive transmission. Moreover, molecular surveillance in domestic animals is essential to clarify their reservoir role in zoonotic filariasis. Collaborative research under a One Health framework is strongly recommended to support Indonesia's goal of lymphatic filariasis elimination by integrating ecological, molecular, and public health strategies.

ACKNOWLEDMENT

The authors wish to express sincere gratitude to the Health Departement of Banyuasi Regency, South Sumatra, Indonesia for all support of this study. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors have read and approved the final version of the manuscript and agree with its submission. This study did

not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research activities were supported by institutional resources from the Health Departement of Banyuasin Regency, South Sumatra, Indonesia

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