



A Descriptive Study of Microfilarial Periodicity Based on the Timing of Blood Sample Collection among Filariasis Patients

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Abstract

Filariasis is an infectious disease caused by filarial worms and transmitted through mosquito bites, with clinical manifestations and transmission closely related to the periodicity of microfilariae in peripheral blood. This study aims to determine the density of *Brugia malayi* microfilariae at different blood sampling times and to identify the predominant type of microfilaria periodicity in the endemic area of Luwuk Bunter Village, East Kotawaringin Regency. This study was a descriptive observational study with a longitudinal (time series) approach, using capillary blood samples from 17 patients confirmed to have filariasis, collected every four hours over a 24-hour period (total sampling). Microfilariae density was calculated per 60 μ L of blood in Giemsa-stained thick blood smears, then analyzed descriptively in terms of mean, standard deviation, minimum value, and maximum value for each sampling time point. The highest average density of *B. malayi* microfilariae was observed at 4:00 a.m. WIB (31.65 ± 60.592 ; range 0–228), followed by 12:00 a.m. WIB (23.53 ± 39.789), then decreased from 08:00 WIB to a low of 12:00 WIB (4.06 ± 9.934). This pattern indicates that *B. malayi* in Luwuk Bunter Village exhibits a nocturnal subperiodic rhythm, with microfilarial density increasing from night through early morning but remaining detectable during the day. *B. malayi* microfilariae in Luwuk Bunter Village, East Kotawaringin Regency, exhibit a nocturnal subperiodic pattern with peak density at 04:00 WIB, which is important as a basis for determining the optimal time for blood sampling in filariasis surveillance activities.

Introduction

Filariasis, or lymphatic filariasis, is a tropical parasitic disease transmitted by mosquito vectors that affects the human lymphatic system (Sinha et al., 2023; de Souza & Bockarie, 2025; Waje et al., 2024). The main parasites causing filariasis are *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, with vector mosquitoes from the genera *Mansonia*, *Anopheles*, *Culex*, *Aedes*, and *Armigeres* (Paisal et al., 2019; Pratiwi et al., 2021). The national prevalence of filariasis in Indonesia is recorded at 1.5%, with Central Kalimantan Province being one of the regions with the highest case rates (2.7%) (Indonesian Ministry of Health, 2024). The village of Luwuk Bunter, in East Kotawaringin Regency, was the focus of a microfilaria prevalence evaluation survey (Pre-Transmission Assessment Survey/preTAS) conducted by the Ministry

of Health in December 2024. Of the 325 samples examined, 17 tested positive for *B. malayi* microfilariae, resulting in a microfilaria rate of 5.23%; thus, this village met the criteria for inclusion in the Mass Drug Administration (MDA) program for filariasis (Indonesian Ministry of Health, 2024).

Based on previous studies, there are four types of microfilaria periodicity: nocturnal periodic, diurnal periodic, nocturnal subperiodic, and aperiodic (Binkienė et al., 2024). Nocturnal periodicity is characterized by very high microfilariae density at night and almost none during the day; this pattern is common in *W. bancrofti*, *B. malayi*, and *B. timori*. Nocturnal subperiodicity is characterized by higher density at night but is still partially detectable during the day, and this pattern is frequently observed in *B. malayi*. If microfilarial densities during the day and night do not differ significantly, this pattern is called aperiodic (WHO, 2013). Understanding these types of periodicity is important because it is directly related to the optimal timing of blood sampling during filariasis surveys to avoid false-negative results (Tan et al., 2026; Asare et al., 2024; Stolk et al., 2022).

The method commonly used to determine microfilaria periodicity is the Aikat and Das method, which assumes that microfilaria density follows a regular pattern and that the data are normally distributed; thus, it is less appropriate for use with fluctuating data distributions (Aikat & Das, 1977; Edyansyah et al., 2021). The novelty of this study lies in the use of a descriptive statistical approach that can describe variations in microfilaria density without relying on the assumption of data normality, making it more suitable for application to irregular distributions of microfilaria density (Field, 2018; Sumbah et al., 2024). This study aims to determine the pattern of *B. malayi* microfilaria density at different blood sampling times and to identify the type of microfilaria periodicity in the endemic area of Luwuk Bunter Village, East Kotawaringin Regency, as a basis for determining the optimal timing for blood sampling in filariasis surveillance activities.

Methods

Study Design and Setting

This study employed a quantitative descriptive observational design with a longitudinal time-series approach to characterize changes in *Brugia malayi* microfilarial density according to the timing of peripheral blood collection. Repeated blood measurements were obtained from the same participants at predetermined intervals over a 24-hour period. The study was conducted in Luwuk Bunter Village, East Kotawaringin Regency, Central Kalimantan, Indonesia, with laboratory examination performed at the East Kotawaringin Regency Regional Health Laboratory. Data collection was conducted from July to August 2025.

Study Population and Participants

The study population consisted of individuals who had been identified as positive for microfilariae during the Ministry of Health pre-Transmission Assessment Survey (preTAS) conducted in December 2024. Of 325 individuals examined during the survey, 17 were positive for *B. malayi* microfilariae, and all 17 eligible individuals were included using a total sampling technique. Medihealtico 7 (4) 966- (5081)

Participants were eligible if they resided in Luwuk Bunter Village, had no history of residing in another filariasis-endemic area during the preceding year, had been confirmed as microfilaria-positive in the December 2024 survey, and had not received antifilarial treatment at the time of blood collection. Participants who did not complete the scheduled series of blood collections were excluded from the study.

Blood Sampling Procedure

Capillary blood samples were collected from each participant at six time points at four-hour intervals within a 24-hour observation period: 16:00, 20:00, 00:00, 04:00, 08:00, and 12:00 Western Indonesian Time (WIB). Thus, each participant underwent repeated blood collection at the same six predetermined sampling times.

Before blood collection, the fingertip was disinfected using 70% alcohol and punctured with a sterile blood lancet. A total of 60 μL of capillary blood was placed on a glass slide in three lines of 20 μL each to prepare a thick blood smear. The smears were subsequently processed and stained with Giemsa solution for 30 minutes, washed, dried, and prepared for microscopic examination following the laboratory procedure described by Dewi (2018). The same sampling and examination procedures were applied at each observation time to ensure comparability of microfilarial counts across the 24-hour period.

Microscopic Examination and Microfilarial Identification

Each prepared blood smear was examined microscopically, beginning at low magnification for general orientation, followed by systematic examination of the entire smear using a zig-zag scanning technique to minimize the possibility of missing microfilariae. The total number of microfilariae observed on each slide was recorded and expressed as the number of microfilariae per 60 μL of blood.

Microfilariae were morphologically identified as *B. malayi* based on characteristic microscopic features, including the presence of a sheath, a relatively long cephalic space, a compact arrangement of somatic nuclei, and two distinct terminal nuclei at the tip of the tail. These morphological characteristics were used consistently for species identification across all examined samples. This clarification is important because the Results explicitly report that all microfilariae detected in the 17 participants were identified as *B. malayi*. Medihealtico 7 (4) 966- (5081)

Measurement of Microfilarial Density and Periodicity

Microfilarial density was defined as the total number of *B. malayi* microfilariae detected in 60 μL of capillary blood at each sampling time. Individual microfilarial counts were recorded separately for each participant at the six observation times to evaluate temporal fluctuations throughout the 24-hour period.

The periodicity pattern was determined descriptively by comparing the distribution of microfilarial density across the six sampling times. A nocturnal subperiodic pattern was interpreted as a pattern in which microfilarial density increased during nighttime and early morning hours but remained detectable during daytime sampling. This operational interpretation was applied to the observed temporal distribution rather than relying on a mathematical periodicity model.

Statistical Analysis

Data were analyzed using SPSS version 20 for Windows. Microfilarial counts for each participant were initially tabulated according to sampling time. For each of the six observation times, descriptive statistics were calculated, including the mean, standard deviation, minimum value, and maximum value of microfilarial density per 60 μL of blood.

Temporal changes in microfilarial density were subsequently compared descriptively across the six sampling times to identify the period of highest and lowest average density and to characterize the overall periodicity pattern. No inferential hypothesis testing was performed because the primary objective of the study was to describe the temporal distribution and periodicity of *B. malayi* microfilariae in the study population.

Result and Discussion

All microfilariae found in the 17 study samples were of the species *B. malayi*, characterized by a thin sheath conforming to the body's contours, a long cephalic region with a length-to-width ratio of approximately 2:1, a body consisting of a tightly packed row of nuclei, and two separate terminal nuclei at the tail end a distinguishing feature from other microfilaria species.

The number of microfilariae in each subject varied depending on the time of sampling (Table 1). The highest density was recorded at 4:00 a.m. WIB at 228 microfilariae (Subject 12), while the lowest density was zero in several subjects at all observation times. Most subjects showed an increase in the number of microfilariae from night through early morning, followed by a decline again toward noon.

Table 1. Microfilaria Density per 60 μ L of Blood for Each Subject Based on Blood Sampling Time (WIB)

Subject Code	4:00 PM	8:00 PM	12:00 a.m.	4:00 a.m	08:00 a.m	12:00 p.m
Bachelor's Degree	0	0	0	2	0	0
Master's	2	18	27	42	15	1
S3	0	0	0	0	0	0
S4	12	42	54	87	42	8
S5	15	39	48	33	15	7
S6	1	6	15	16	12	1
S7	1	3	5	3	3	0
S8	30	36	101	114	59	12
S9	1	3	6	4	0	0
S10	0	0	0	0	0	0
S11	0	0	0	0	0	0
S12	14	123	135	228	210	40
S13	4	8	9	9	0	0
S14	0	0	0	0	1	0
S15	0	0	0	0	0	0
S16	0	0	0	0	0	0
S17	0	0	0	0	0	0

Note: WIB = Western Indonesian Time; S = Subject

The highest average microfilaria density was recorded at 4:00 a.m. WIB (31.65 ± 60.592 ; range 0–228), followed by 12:00 a.m. WIB (23.53 ± 39.789), while the lowest average was recorded at 12:00 WIB (4.06 ± 9.934 ; range 0–40). At other times, the average density ranged from 4.71 to 21.00 (Table 2). This pattern indicates that microfilaria density tends to increase at night, peaks in the early morning, and then decreases again toward noon.

Table 2. Mean, Standard Deviation, Minimum, and Maximum Values of Microfilaria Density by Sample Collection Time

Sampling Time (WIB)	Mean $\pm$ Standard Deviation	Minimum Value	Maximum
4:00 PM	4.71 ± 8.335	0	30
20.00	16.35 ± 31.231	0	123
00.00	23.53 ± 39.789	0	135
04.00	31.65 ± 60.592	0	228
8:00	21.00 ± 51.510	0	210
12:00	4.06 ± 9.934	0	40

Note: WIB = Western Indonesian Time

The highest average number of microfilariae was found at 4:00 a.m. WIB, followed by midnight, and then decreased between 12:00 p.m. and 4:00 p.m. WIB. This pattern indicates that *B. malayi* in this study exhibits nocturnal subperiodic periodicity, with microfilariae concentrations in peripheral blood peaking at night through the early morning, though they remain detectable in the morning and afternoon (Kleinhapl et al., 2026; Juliana et al., 2025; Shea et al., 2026).

The consistently high microfilaria density at 8:00 a.m. WIB differs from that of purely periodic strains, which typically decline sharply in the early morning, indicating a deviation from the purely periodic pattern. This phenomenon can be explained by the biological characteristics of subperiodic strains, which often involve zoophilic reservoirs, allowing microfilariae to circulate longer in the human bloodstream (Mathison et al., 2019; Tan et al., 2016). The host's circadian regulation including the effects of melatonin on microfilaria migration, variations in sleep rhythms, hormonal responses, and physiological factors such as body temperature and circulatory dynamics is thought to also slow the return of microfilaria to internal organs (Mallawarachchi et al., 2018; Paisal et al., 2019). These findings indicate that the microfilaria population in Luwuk Bunter Village has adapted to the activity of local vectors, which are active from night through the early morning (Mbewe et al., 2025; Mwesige et al., 2025; Vajda et al., 2024).

These results are generally consistent with a study in Muaro Jambi Regency, which found variations in periodicity, with three samples exhibiting a nocturnal pattern and two samples exhibiting a subperiodic nocturnal pattern (Supranelfy et al., 2019). In contrast, a study in West Sulawesi reported that the behavior of *B. malayi* microfilariae is entirely nocturnal periodic (Ramadhani et al., 2019), differing from the results of this study, which showed that microfilariae were still found during the day even though the highest density occurred at night. This difference is likely due to variations in *B. malayi* strains and ecological conditions in each study area.

Another study in Pekalongan City concluded that *W. bancrofti* exhibits a nocturnal periodic pattern,¹² which differs from the findings of this study, which indicate a nocturnal subperiodic pattern. This suggests differences in characteristics among filarial species, with *W. bancrofti* tending to be purely nocturnal, while *B. malayi* exhibits greater variability with a subperiodic tendency. The subperiodic pattern provides a biological advantage for the parasite to persist in the blood during the day, even in small numbers, thereby increasing the chances of transmission via vectors with varying biting activity (Khan et al., 2015; Braks et al., 2026; Ayisi et al., 2026).

The difference between the nocturnal subperiodic pattern observed in this study and the pure nocturnal pattern observed in previous studies may be influenced by several factors: biological factors, such as variations in *B. malayi* strains in Indonesia, which are known to exhibit two types of periodicity (nocturnal and subperiodic); host factors such as sleeping habits, immunological status, and bednet compliance; environmental factors such as temperature and humidity that influence the vector's activity period; and methodological factors such as sampling intervals and the number of positive samples (Khan et al., 2015). This study contributes epidemiological data on filariasis in Central Kalimantan, particularly in endemic areas that have not been widely reported in the literature, and is relevant for the development of surveillance and control strategies for filariasis in areas characterized by nocturnal subperiodic periodicity (Pratiwi et al., 2026; Gunaratna et al., 2026; Banerjee & Kaushal, 2026).

This study has several limitations, namely the relatively small number of subjects and the fact that they were recruited from only one endemic village; therefore, the results may not necessarily represent variations in microfilaria periodicity in other endemic areas with different

ecological conditions or vectors (Kamgno et al., 2025; M'Bondoukwé et al., 2026; Adeshina et al., 2026). In addition, variations in temperature, humidity, and individuals' daily activities (e.g., sleep patterns and exposure to mosquitoes) could potentially influence microfilaria density; however, not all of these factors could be controlled during the sampling period.

Conclusion

B. malayi microfilariae in filariasis-positive subjects in Luwuk Bunter Village exhibited a nocturnal subperiodic pattern, with peak density at 4:00 a.m. WIB and the lowest level at 12:00 p.m. WIB. This pattern indicates that *B. malayi* microfilariae in the region have adapted to the activity of local vectors, which tend to be active from night through the early morning, while their continued detection during the day demonstrates the parasite's biological flexibility in maintaining its transmission cycle. These findings can serve as a basis for local health departments and community health centers in determining the optimal timing for blood testing and filariasis surveillance activities, as well as for the national filariasis elimination program in adapting control strategies to local variations in the behavior of microfilariae and their vectors. Further research with a larger sample size, more frequent sampling intervals, and measurements of environmental factors is recommended to strengthen the analysis of microfilaria periodicity in endemic areas.

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