Original Research Article

Survey of filariasis and Microfilarial periodicity in Musi Rawas District, South Sumatra, Indonesia

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ABSTRACT

Background: Lymphatic filariasis is a parasitic disease that is similar to the threads of its habitat in the lymph system that infect humans, namely Wuchereria bancrofti, Brugia malayi, and Brugia timori. Although B. malayi commonly infects humans, recent evidence also suggests that Brugia pahangi, an afilarial nematode naturally found in cats, can cause clinical infection in humans, with clinical features consistent with lymphatic filariasis.

Methods: Cross-sectional with an observational and analytic approach. The results of a positive microscopic examination were carried out by Brugia Rapid Test for B. malayi and PCR examination for B. malayi and B. pahangi. Positive microscopic results were then checked for periodicity of microfilariae every 2 hours for 24 hours

Results: From the research, 17 people were positive for B. malayi microfilariae (mf rate 6.34%). The Brugia Rapid Test had 17 positive results. PCR results of 14 people were positive/formed a band at 322 bp. The results of the sample sequencing were B. malayi species. PCR results of B. pahangi were not found to be positive / band formed in all samples. The periodicity results of microfilaria peaked at 00:00-04:00 with the nocturnal periodic type.

Conclusions: Lubuk Pauh Village, Musi Rawas Regency is still endemic for malayi filariasis with a high level of endemicity and is not an endemic area for filariasis pahangi. The periodicity of microfilariae indicates a nocturnal periodic type.

Keywords: B. malayi, B. pahangi, Periodicity microfilariae

INTRODUCTION

Lymphatic filariasis is a parasitic disease caused by three species of thread-like worms whose habitat is in the human lymph system. Lymphatic filarial worms that infect humans are Wuchereria bancrofti, Brugia malayi, and Brugia timori. Lymphatic filariasis is found throughout the world, especially in tropical and some sub-tropical areas. In October 2018 an estimated 856 million people in 52 countries around the world are at risk of contracting filariasis. It is estimated that 60% of all cases are in Southeast Asia. In 2004 more than 120 million people were infected with filariasis.¹³

Although B. malayi commonly infects humans, recent evidence also suggests that B. pahangi, an afilarial nematode naturally found in cats, can cause clinical infection in humans, with clinical features consistent with lymphatic filariasis. This suggests that B. pahangi infection may be more common in humans than previously thought.⁴⁻⁶

Microscopic examination to find microfilaria is the gold standard examination for the diagnosis of filariasis. Immunology-based examinations can be carried out regardless of the periodicity of microfilariae, which aims to detect parasite antibodies or antigens, including detection of filarial antibodies with the Brugia Rapid Test.⁵
Polymerase chain reaction (PCR) examination is used to identify Deoxyribose nucleic acid (DNA) lymphatic filarial parasitic worms in the human body. Chronic sufferers are a source of filariasis transmission if microfilariae are still found in the peripheral blood. The results of microscopic examination of chronic patients rarely find filarial worms in the peripheral blood, so it is necessary to examine chronic patients using the PCR method.7

*B. malayi* is a periodic nocturnal strain, its microfilariae can only be detected from the peripheral circulation between late afternoon and midnight. Meanwhile, subperiodic nocturnal strains of microfilariae can be found in the peripheral circulation throughout the day, but the peak is at night, zoonotic and transmitted to humans (via mosquitoes) from cats and wild carnivores as reservoir hosts.78 The research objective was to determine the endemicity of filariasis and periodicity of microfilaria in Lubuk Pauh Village.

**METHODS**

This type of research is cross-sectional with observational and analytic approaches.9 The research site was conducted in Lubuk Pauh Village, Bulang Tengah Suku (BTS) Ulu District, Musi Rawas Regency, South Sumatra Province. The time of the research was carried out in 2018-2019. The population's finger blood samples that can be taken are 268 people. Examination of blood samples was carried out by the microscopic method of *B. malayi* and *B. pahangi* species. Finger blood samples were taken at night starting at 20.00 WIB based on filarial worms in Indonesia at night and the results were: positive or negative.

The calculation is done by the formula MOH:10

\[
\text{Mf} = \text{rate} = \frac{\text{the number of positive blood stains for microfilariae}}{\text{the blood count was checked} \times 100\%}
\]

Positive microscopic examination results were carried out by Brugia Rapid Test for *B. malayi* species and PCR examination for *B. malayi* and *B. pahangi* species. A positive microscopic result was then checked for periodicity of microfilariae every 2 hours for 24 hours.

Detection of *B. malayi* antigen using the Brugia Rapid Test in accordance with the method of action issued by the product manufacturer Rezson Diagnostics International.

**PCR examination**

Samples derived from scrapings from positive sample preparations. Reagents: Genomic DNA Mini Kit (tissue) from the Geneaid brand. To detect the *B. malayi* gene, a running PCR process was carried out using a master mix reagent with forward HhaI primers (5’GGCATAAATTCATCAGC-3’) and reverse HhaII primers (5’GGCGAAACTTATTACAAAAAGC-3’).

Optimization temperature used for running PCR is 95OC activation for 5 minutes, denaturation 94OC for 30 seconds; annealing 48OC 40 seconds; extension 72OC 1 minute; with 35 cycles, final extension of 72OC 5 minutes (additional from researchers); and 25OC for ∞. Reading and analysis of positive electrophoresis results if a band is formed at 322 bp in the DNA sequence.11

PCR examination to detect species *B. pahangi* with forward primer 5’TATTGCCTGTTATGC3’ and reverse primer 5’TGTATATGTGATGAC3’, with an optimization temperature of 95OC 10 minutes, 94OC 1 minute; 54OC 1 minute; 72OC 2 minutes; 30 cycles, 72OC 10 minutes. Readings and analysis of positive electrophoresis results if a band is formed at 633 bp in the DNA sequence.12

**The periodicity of microfilariae**

Samples of microfilaria periodicity: people who were willing to have their finger blood samples taken every 2 hours for 24 hours were 4 positive sufferers of high density microfilariae.

Analysis of the periodicity of microfilaria in the statistical analysis by Aikat and Das.13 This statistical analysis provides an overview of the variation in the density of microfilariae in the peripheral blood of patients over time. The relationship between the density or the number of microfilariae is expressed in (Y), the number of samples is expressed in (n), the average number per blood draw is expressed in (m), the time of blood collection is expressed in a price (h), the peak time of density in blood (K). The relationship between (Y) and (h) can be expressed in the following equation:

\[
Y = m + b \cos 15h + c \sin 15h
\]

\[
m = y \div n
\]

\[
b = 2 \left( \sum y \cos 15h \right) \div n
\]

\[
c = 2 \left( \sum y \sin 15h \right) \div n
\]

thus, the prices a and D (periodicity index) can be calculated using the following formula:

\[
a = \sqrt{b^2 + c^2}
\]

\[
D = a + m
\]

a value of D greater than 50 indicates the nature of periodic harmonic waves, whereas if it is less it shows subperiodic (nocturnal or diurnal) or non-periodic, while for periodic type the value of D is greater than 50 or close to 100%. From the above equation, the peak density of microfilariae (K) can be calculated by the following formula:

\[
\tan 15K^0 = c \div b
\]
The cycardian rhythm can be calculated by the formula:

\[ F = \frac{(n + 2)a^2}{(n^2 - (n + 3))(\sum y^2 - (\sum y)^2 - n - ((n + 2)a^2)^2)} \]

The variation in density of microfilariae in peripheral blood in the case of periodicity examination results shows cicardian properties if the calculated F value is greater than the theoretical F value or F table (can be seen in the statistical table).

**RESULTS**

Microscopic examination of microfilariae, Brugia Rapid Test, and PCR in Lubuk Pauh Village (Table 1).

<table>
<thead>
<tr>
<th>ID*</th>
<th>Results</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Mikroskopis</strong></td>
<td><strong>Brugia Rapid Test</strong></td>
</tr>
<tr>
<td></td>
<td><em>B. malayi</em></td>
<td><em>B. pahangi</em></td>
</tr>
<tr>
<td>1</td>
<td>Positive/+3</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Positive/+27</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Positive/+6</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Positive/+6</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Positive/+2</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Positive/+4</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Positive/+3</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>16</td>
<td>Positive/+94</td>
<td>Negative</td>
</tr>
<tr>
<td>17</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
<tr>
<td>18</td>
<td>Positive/+91</td>
<td>Negative</td>
</tr>
<tr>
<td>19</td>
<td>Positive/+1</td>
<td>Negative</td>
</tr>
</tbody>
</table>

From the Table 1, of 19 people, the microscopic examination was carried out, the results showed that 17 people were positive for *B. malayi* microfilaria (mf rate 6.34%), so the level of endemicity was still high for the size of one village with a rate of >1%. From the 17 positive microscopic results, the Brugia Rapid Test was examined and 17 were positive. PCR results of 14 people were positive/formed a band at 322 bp. The results of the sample sequencing were *B. malayi* species. PCR results of *B. pahangi* were not found to be positive/band formed in all samples.

In Figure 1, it can be seen that the positive results/band formation at 322 bp in the blood sample of the population of Lubuk Pauh Village using the *B. malayi* primer as many as 14 samples (Figure 1).

From the results of the Table 2, examination of the periodicity of microfilariae in peripheral blood was 12 times taken for 24 hours, the Y value or the total number was mostly found as many as 224 individuals, namely in patients with code 16, an average of 18.83 individuals per examination (60 µl of blood). On the other hand, the lowest density of patients with code 5 was 24 tails, an average of 2 animals per examination (60 µl of blood) (Table 2).

The microfilaria density for 24 hours can be seen in the graph below (Figure 2).

**Table 2: Microfilariae periodicity examination.**

<table>
<thead>
<tr>
<th>Hours of Blood Collection (WIB*)</th>
<th>Microfilariae Density (mf/60 µl of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ID 2</strong></td>
<td><strong>ID 5</strong></td>
</tr>
<tr>
<td>18:00</td>
<td>4</td>
</tr>
<tr>
<td>20:00</td>
<td>1</td>
</tr>
<tr>
<td>22:00</td>
<td>10</td>
</tr>
<tr>
<td>24:00</td>
<td>2</td>
</tr>
<tr>
<td>02:00</td>
<td>10</td>
</tr>
<tr>
<td>04:00</td>
<td>3</td>
</tr>
<tr>
<td>06:00</td>
<td>1</td>
</tr>
<tr>
<td>08:00</td>
<td>0</td>
</tr>
<tr>
<td>10:00</td>
<td>0</td>
</tr>
<tr>
<td>12:00</td>
<td>1</td>
</tr>
<tr>
<td>14:00</td>
<td>1</td>
</tr>
<tr>
<td>16:00</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total (Y)</strong></td>
<td>41</td>
</tr>
</tbody>
</table>

*WIB: Waktu Indonesia Barat
In Figure 2, it can be seen that microfilariae start to circulate a lot in the peripheral blood at 18:00 WIB (afternoon) and reach their peak at night (20:00-22:00) WIB and the decrease in density begins at 06:00-12:00 WIB and rises again at 16:00 WIB.

From the results of microfilaria calculations, statistical analysis was carried out using the Aikat and Das formulas. The results of the periodicity statistical analysis in Lubuk Pauh Village can be seen in the Table 3.

Table 3: Statistical analysis on the results of Microfilariae periodicity examination by using the Aikat and Das formula.

<table>
<thead>
<tr>
<th>Statistic Analysis</th>
<th>ID 2</th>
<th>ID 5</th>
<th>ID 15</th>
<th>ID 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>41</td>
<td>24</td>
<td>224</td>
<td>91</td>
</tr>
<tr>
<td>Y^2</td>
<td>279</td>
<td>94</td>
<td>7638</td>
<td>1057</td>
</tr>
<tr>
<td>Y cos 15 h</td>
<td>15,4540</td>
<td>8,4640</td>
<td>103,914</td>
<td>16,4900</td>
</tr>
<tr>
<td>Y sin 15 h</td>
<td>-8,6960</td>
<td>-11,4640</td>
<td>-31,4900</td>
<td>-21,9540</td>
</tr>
<tr>
<td>M</td>
<td>3,4166</td>
<td>2</td>
<td>18,8333</td>
<td>7,58</td>
</tr>
<tr>
<td>B</td>
<td>2,5756</td>
<td>16,928</td>
<td>17,319</td>
<td>2,7483</td>
</tr>
<tr>
<td>C</td>
<td>-1,4493</td>
<td>-1,9106</td>
<td>-5,2483</td>
<td>-3,659</td>
</tr>
<tr>
<td>A</td>
<td>2,9554</td>
<td>17,035</td>
<td>18,0965</td>
<td>4,5762</td>
</tr>
<tr>
<td>K</td>
<td>00.23'01</td>
<td>00.25'12</td>
<td>01.13'12</td>
<td>03.31'48</td>
</tr>
<tr>
<td>F</td>
<td>4,51</td>
<td>-9,24</td>
<td>12,44</td>
<td>4,68</td>
</tr>
<tr>
<td>D</td>
<td>86,50</td>
<td>85,17</td>
<td>96,08</td>
<td>60,37</td>
</tr>
</tbody>
</table>

From the table above the results of statistical analysis according to the Aikat and Das formula show that the peak density of microfilaria (K) is a code 2: 02.03'00", code 5: 00.25'12", code 16: hour 01.13'12", and code 18: 03.31'48". The value of F code 2 (4,51>4,26) indicates a cicardian rhythm (periodic), the value of F code 5 (-9,24<4,26) indicates an uncicardian rhythm (non-periodic), the value of F code is 16 (12, 44>4.26) indicates a cicardian (periodic) rhythm, and the value of F code 18 (4.68>4.26) indicates a cicardian (periodic) rhythm.

DISCUSSION

The number of positive residents according to researchers who saw directly in the field was because the population had not implemented the filariasis treatment program properly and correctly, such as only in the early years that were supervised by officers, then no longer, the distance from the houses at night, residents can stay in the forest for ±1 week, the environment is less clean, most of the houses do not use wire netting, most of the residents sleep without using a mosquito net (results from direct interviews at the time of blood sampling), and the residents’ habit of living outside the house until late at night.

The results of the positive population for microfilaria on microscopic examination and the Brugia Rapid test but on negative PCR examination, this is probably due to the low density of microfilariae in the infected population’s blood. There is a population of chronic sufferers on microscopic examination and the Brugia Rapid Test results are negative but on the PCR examination it is still positive, this is probably because the patient is infected with one sex of B. malayi, the number/density of microfilaria in the blood is too low so that it is difficult to detect.

According to McNulty et al a diagnostic technique based on direct detection of microfilariae in the blood can be
insensitive when the microfilariae are no longer in the peripheral blood circulation or at very low microfilariae concentrations.\textsuperscript{14} According to Soeyoko the asymptomatic microfilaraemia group (no microfilariae in the blood or no clinical symptoms of filariasis) was not found in the blood due to several factors, namely the patient is still in the prepatent stage, the patient is infected by one sex \textit{B. malayi}, the number/density of microfilariae in the blood is too low so that it is difficult to detect, or the emergence of an immune response especially against microfilaria is excessive (for example in Occult filariasis).\textsuperscript{19}

According to Fischer et al serological tests can be efficiently combined with PCR-based assays, to overcome the main drawbacks of each method. The \textit{Brugia} Rapid Test is a rapid screening test that can be used on blood samples today and can be performed in the field. Unfortunately, these serologic and other tests provide only indirect estimates of infection prevalence, because the \textit{Brugia} Rapid Test cannot differentiate between microfilaraemia and microfilaremia or between persistent and recently treated infections. If high sensitivity is to be achieved, the requirement for a relatively large number of day or night blood samples is drawn. In general, the presence of microfilariae is associated with the relatively high anti-filarial IgG4 titers. Detection of filarial DNA in blood samples requires a relatively large amount of venous blood. In any area where \textit{brugia} filariasis is being investigated, screening for anti-filarial IgG4 with the \textit{Brugia} Rapid Test will be the first choice, to identify areas where the disease may be endemic. Subsequent use of PCR-based tests will help to identify microfilaraemia individuals and subjects who, due to cross-reactivity, were false positives in the \textit{Brugia} Rapid Test survey. The combined data from the \textit{Brugia} Rapid Test and PCR-based assays should provide a relatively accurate picture of the local transmission intensity. Techniques based on molecular biology will also allow \textit{B. malayi} to be distinguished from \textit{B. timori}. The combination of the serologic and PCR-based tests described above may be useful for efficient and sensitive detection of \textit{B. malayi} causes. Using two tests simultaneously allows the drawbacks of each test to be overcome.\textsuperscript{16}

Research Chai et al out of a total of 378 residents, 6 (1.6\%) were positive for \textit{B. malayi} microfilariae. The presence of 6 cases of microfilaraemia indicates an active life cycle prevalence of \textit{B. malayi} microfilaraemia in these islands.\textsuperscript{17} The most important community-related uncertainty is the issue of compliance. It will be valuable to develop “compliance profiles” of communities to identify those groups of individuals who remain “persistently non-compliant” during MDAs (example, children, upper socioeconomic classes, young men, older ages), and then to determine the causes of this noncompliance and effective approaches to overcoming it.\textsuperscript{18} According to Haarbrink et al cit Putri microfilaria count on microscopic examination is the gold standard diagnosis for filariasis. But this method is less effective, because of the difficulty of taking blood samples at night, and inadequate

sensitivity, and less sensitivity in patients with tropical pulmonary eosinophilic and elephantiasis.\textsuperscript{19}

The presence of patients with clinical filariasis (acute or chronic), the more suspected filariasis transmission. The presence of new filariasis sufferers also strengthens the suspicion of filariasis transmission in the area. The presence of people who are found to be positive for microfilaria in their blood, the presence of a high mf rate, further strengthens the suspicion of active transmission.\textsuperscript{20}

According to research conducted by Fischer et al in Central Sulawesi, from taking 113 samples of day blood, the results were 37 positive samples, 76 negative microfilariae samples. Of the 37 positive samples, PCR checked, the results were 31 positive and 6 negative. The 6 PCR negative results obtained from infected individuals came from people with a low density of microfilariae in their blood. From the negative results of 76 microfilariae examined by PCR, 3 samples were positive.\textsuperscript{21}

Research by Santoso et al in 4 villages in Muara Sabak District, Tanjung Jabung Jambi Regency, from a sample of 25 patients whose blood was examined for PCR, including 18 chronic sufferers, 6 positive patients, and 1 chronic and positive microfilaria patient from the results of DNA isolation after samples were found. positive PCR results were then sequenced. The sequencing results of 8 positive samples of microfilaria DNA obtained 6 positive samples of \textit{B. malayi} and 2 positive samples of \textit{B. timori} which need further investigation.\textsuperscript{15}

A positive \textit{Brugia} Rapid Test result cannot be a single determinant for a clinical decision that the species found is \textit{B. malayi} but must look at the results of microscopic examination by looking at the morphology of the microfilaria and PCR sequencing results to determine the species found. The results of this study can be used as a reference that samples of blood preparations for examination of malayi filariasis that have been stained with Giemsa can be used for 2 examinations, namely microscopic examination, and PCR. DNA samples isolated from blood preparations can be used as samples for PCR examination by means of scraping blood samples using a sterile scalpel.

The periodicity of microfilariae which are periodic nocturnal, the stage of microfilariae is found in peripheral blood, especially at night and reaches its peak at 22.00-01.00, while microfilariae, which have subperiodic nocturnal characteristics, are in the peripheral blood for 24 hours but reach their peak at 18.00-22.00. In non-periodic microfilariae, the stage of microfilariae can be found in the peripheral blood at any time and never reaches a peak.\textsuperscript{21,22}

Based on the theory of periodicity types of microfilariae which are periodic nocturnal, the microfilaria stage is found in the peripheral blood, especially at night, and reaches its peak at 22:00-01:00. From the results of this study, it means that the periodic nature of the peak
nocturnal cannot always be ascertained at that hour because the results obtained the peak periodicity of microfilaremia at 00:00-04:00 and possibly this is also due to variations in the time the mosquito vectors suck blood.

The periodic rhythm of variation (F) is more dominant, but there is 1 patient with code 5 who is non-periodic. It is not necessary to say that the patient is a separate group from other cases, patients with code 5 are guaranteed to remain in the same group. The "non-periodic" rhythm in patients with Code 5 that seems to be seen as a variant of the more dominant periodic, especially considering the peak density of microfilariae (K) is obtained at night at 00:25'12".

Generally, non-periodic, subperiodic, or periodic nocturnal characteristics of B. malayi or filaria microfilariae are not individual characteristics but population characteristics, as well as adaptation of parasites to the biting behavior of the infectious mosquito vector. In natural selection, basically the variance of microfilariae that does not match their behavior so that they do not meet the mosquito vector when biting, certainly cannot spread to other sufferers, this means that they cannot continue their life cycle. The behavior of microfilariae moving actively from visceral blood to peripheral blood must be in accordance with the biting behavior of the mosquito vector so that it can infect the mosquito vector with the blood it sucks so that it can successfully continue its life cycle even though the microfilariae is inhaled in limited quantities.

Judging from which habitat is more suitable, basically, microfilariae prefer visceral blood to peripheral blood, so that their presence is more in visceral blood. In adaptation to mosquito vectors, microfilariae must continuously undergo natural selection so that in the course of time as a whole there can be changes in character and finally a match between parasite behavior in the peripheral blood and mosquito vectors.23

The limitation of sampling for this periodicity is because some people who are positive for microfilaria are not in the village, are not willing to have blood drawn every 2 hours for 24 hours, and some are sick at the time of sampling so that blood sampling is not continued. However, it is hoped that it can describe the periodicity of microfilariae in the area.

According to Sudjadi and Hadianto’s research in the Mahakami Delta of East Kalimantan, the results of 6 microfilariaemia patients of B. malayi were examined for the periodicity of their microfilariae, it turned out that cases with nonharmonic or non-circadian waves were seen to dominate (5 cases), while one other case showed a harmonic wave. circadian, but with peak time at noon, 15.54'00.23

Taking each positive blood sample for microfilariae for the determination of the periodicity of microfilariae must be precise and according to size so that each sample has the same blood volume size so that there is no error in calculating the number of microfilariae. Sampling should be done every 2 hours for 24 hours and patients who are willing to be sampled must be provided with one place/house to make the sampling process easier.

CONCLUSION

Lubuk Pauh Village, Musi Rawas Regency is still endemic for malayi filariasis with a high level of endemicity, and Lubuk Pauh Village is not an endemic area for filariasis pahangi. Microfilaria periodicity shows the periodic nocturnal type with the peak density of microfilariae at 00:00-04:00.

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