The diversity of Anopheles blood feeding patterns suggests different malaria protection strategies in different localities

Irfanul Chakim, Tepanata Pumpaibool

Abstract

Background: Malaria is a significant health burden for many countries worldwide. Insecticide-treated bed nets and mosquito repellent are considered effective methods for preventing Anopheles bites. However, changes in the biological properties of the vector have led to a reduction in their effectiveness. Most published studies have only investigated the human population factor, not the dynamics of vector behavior. Therefore, this study aims to investigate the importance of primary vector activity for selecting an appropriate malaria protection strategy.

Methods: Initially, active case detection (ACD) was carried out in western and eastern parts of Indonesia, Jambi and Sumba, to confirm their endemicity level. According to the 2016 national health report of Indonesia, Jambi has an annual parasite index (API) of 0.14 and Sumba has an API of 5.41. A series of entomological observations were carried out to compare the biting activity of Anopheles vector in two localities, with a total of 216 houses and catchers (108 in each study site).

Results: The results indicated that endemicity at the sub-district level is higher than that at the provincial level. Only Anopheles balabacensi was found to be exophagic. Multiple comparisons found different biting times between the sites, suggesting that early evening (18.00-20.00) is most likely to be the time when mosquitoes transmit the Plasmodium parasite in Jambi, while during sleeping hours (21.00-01.00) is the peak biting time of Anopheles mosquitoes in Sumba.

Conclusions: The study demonstrates the importance of Anopheles species blood feeding patterns in selecting an appropriate malaria protection strategy.

Keywords
Malaria, Anopheles, diversity, blood feeding pattern, protective strategy
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Introduction

Malaria is a disease that is transmitted by female *Anopheles* vectors. Generally, malaria control is achieved by mass deployment of insecticide-treated bed nets (ITNs), treated with insecticide corresponding to the biological activity of the vector. It has been shown that the distribution of ITNs is responsible for a reduction of 68% in malaria burden in sub-Saharan Africa. This control method has been widely distributed and a dramatic increase in use has resulted in the mass utilization of ITNs in many countries. Additionally, personal protection has been found to be effective against mosquito bites and its use has led to a reduction in malaria infection. However, frequent daily application is required in order to ensure its effectiveness.

The efficacy of both protection strategies may be problematic as mosquito behavioral activities differ significantly between locations, as observed in Africa, where the vectors exhibit behavioral plasticity. The shifting behavior of the *Anopheles* vector is a factor that contributes to reduced ITN effectiveness. The behavioral changes of *Anopheles* mosquitoes are in the form of shifts to exophagic behavior and biting time modification. Several findings indicate the ineffectiveness of repellent against malaria infection. The limitations of repellent seem to be related to daily adherence and compliance and disproportional utilization. This issue may be due to the assessment of mosquito protection agents being influenced by social desirability rather than the impact that such types of protection have on the biological property of the vector.

The most effective method of *Anopheles* biting protection varies between sites and is dependent on the biting activity of the vector. In Uganda, intensive use of ITNs has been suggested due to the biting pattern of *Anopheles gambiae*, with biting mostly occurring late at night, during the time the human population is asleep. In contrast, bed nets may not provide proper protection against the same *Anopheles* species in Burkina Faso due to an early evening biting time. Limited studies have investigated *Anopheles* biting patterns in the Indonesian archipelago. Thus, our study aimed to specifically address the information gap of *Anopheles* biological properties in Indonesia.

Methods

Study sites

The sampling was carried out in two localities representing different endemicity areas, namely Jambi province and Sumba Island (Nusa Tenggara Timur Province). Jambi is in Sumatra Island, the western part of Indonesia, geographically situated at 0.45 ° North Latitude, 2.45 ° South Latitude and between 101.10 ° -104.55 ° East Longitude. Sumba Island is situated in the eastern part of Indonesia, with an area of 10,710 km² and coordinates of 9°40’S 120°00’E. Jambi and Sumba have a total population of 3,515,017 and 685,186, respectively. Jambi has 11 districts with 136 sub-districts and Sumba has four districts with 44 sub-districts. From all of the sub-districts over the sites, the sub-district of each area with the highest number of cases of malaria was selected for our study to be carried out in (Lembah Masurai in Jambi and Kodi Balghar in Sumba). According to the 2016 national health report of Indonesia, Jambi has an annual parasite index (API) of 0.14 and Sumba has an API of 5.41 (Table 1).

Parasitological investigation

To investigate the API in each sub-district, a series of parasitological assessments were carried out. This assessment was conducted from November 2017 to July 2018 in Jambi and from May to August 2018 in Sumba. Active case detection (ACD) was carried out daily in each site, performed by a local primary healthcare worker. Only people with a tympanic temperature of more than 37.5°C were included in the study. People were asked to go to the local village office for where the finger prick blood sample was collected. Cases were confirmed by light microscopy and prick blood samples were collected directly onto glass slides. A total of 559 and 500 blood samples were taken from Jambi and Sumba, respectively. Two certified independent microscopists assessed all the slides taken from ACD and determined the parasite species.

The API of both sites was calculated using the following formula:

\[
\text{Annual parasite index} = \frac{\text{total cases/total months of collection} \times 12}{\text{total population in each subdistrict}}
\]

Entomological observation

A series of entomological observations were conducted for comparison of the pattern of blood feeding of the potential vector between the two localities. A 24-day observation was done in each area. The human landing catch (HLC) method was used for obtaining *Anopheles* vectors. The HLC method is a standard method for measuring the exposure of humans to mosquito bites as it directly captures mosquitoes that land and attempt to feed on collectors. HLC requires an indoor and outdoor catcher present over 12 hours, from 6 pm to 6 am, to reflect the pattern of *Anopheles* biting and blood feeding time preference. The catchers were the owners of the houses and were trained on how to conduct the HLC method. In the current study, indoor and outdoor mosquito collection was carried out at each house. Six houses were selected for daily HLC and there

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of malaria cases</th>
<th>Collection time (months)</th>
<th>Population</th>
<th>Yearly incidence rate (sub-district level)</th>
<th>Reported incidence rate (provincial level) [Reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jambi</td>
<td>71</td>
<td>9</td>
<td>26,579</td>
<td>3.56</td>
<td>0.14 [23]</td>
</tr>
<tr>
<td>Sumba</td>
<td>140</td>
<td>4</td>
<td>21,049</td>
<td>15.96</td>
<td>5.41 [23]</td>
</tr>
</tbody>
</table>
were six days of collection per a week. Inclusion criteria of
the houses was as follows: (1) three houses had to have had a
malaria infection during the previous one-year period; (2) the
other three houses had to have had an absence of malaria infec-
tion for at least one-year and had to be in close proximity
to the infected houses. The information about malaria infec-
tions at each house was obtained by interviewing each house
member. In total, there were 216 houses and 216 catch-
ers (108 at each study site). The observation was carried out
24 days in each study site. Random selection was done for rep-
etition (for example, a house which had indoor collection in
the first week would have outdoor collection in the next week
and change to indoor in the last week and vice versa); thus,
each house had the same pattern of an indoor and outdoor
collection. The distance between each house was less than two
kilometers to avoid biases due to potential differences in
mosquito species abundance. All the mosquito species were
confirmed by entomological experts from Eijkman Institute for
Molecular Biology, Jakarta, Indonesia by dissection and view-
ing under a light microscope using the Anopheles identification
key developed by Rattanarithikul et al.29.

Statistical analysis
To analyze the data, descriptive and analytical tests were
carried out to analyze the mosquito blood feeding pattern
of each site. The analysis provided three types of results:
1) the preferred biting time of Anopheles mosquitos at each site
by comparing the number of collected mosquitos in each site
using a student t-test statistical method; 2) a comparison of the
number of mosquitos collected indoors and outdoors from each
location using the Mann Whitney test; and 3) multiple compari-
sons of biting time by pooled analysis for each location using
the Kruskal-Wallis test and Dunn’s multiple comparison test.

All the analyses and comparisons were carried out using Graph-
Pad Software version 8.00 (La Jolla California, USA). Relative
abundance and human landing rate (HLR) were calculated
using the following formulas:

Relative abundance = \frac{\text{Total mosquitos collected of each species} + 100}{\text{Total mosquito collection}}

Human landing rate = \frac{\text{Total mosquitos collected of each species}}{\text{Total number of catchers}}

Ethical statement
Informed consent was obtained from collectors perform-
ing HLC. Permission was also received from the owner of the
house and the community on both sites. Community permis-
sion has been obtained by collectively gathering village residents
along with the head of the village in the village office. Written
informed consent was also sought for every participant of the
parasitological assessment. This study was approved by the
ethics commission of Universitas Muhammadiyah Semarang
[22/EC/FKM/2017].

Results
The parasitological assessment found a total of 211 cases of
malaria in both localities30. Only Plasmodium vivax was found
in Jambi, responsible for 71 malaria cases. Participants from
Jambi were 60.6% male (43) and 39.4% female (28) with a
mean age of 15.5 years, ranging from one to 59 years. In Sumba,
three types of Plasmodium were successfully detected during
ACD. From a total of 140 malaria cases in Sumba, 92 (65.7%)
were Plasmodium falciparum, 43 (30.7%) were Plasmodium
vivax, and 5 (3.6%) were Plasmodium malariae. Participants
from Sumba were 58.6% male (82) and 41.4% female (58) with
a mean age of 10.9 years, ranging from one to 53 years. The
calculated APIs of the two study sites were 3.56 and 15.96,
respectively (Table 1). The API result of this study is different
to the national health report of the Ministry of Health, Indonesia.
The API is up to 2.95-25.4-fold higher at the sub-district level,
found in this report, than at the provincial level, as stated in the
report.

A total of 2,435 Anopheles mosquitos were successfully collec-
ted from 216 houses and 216 catchers at the two locations
(108 houses and catchers at each study site)29. There was a sta-
tistical difference in the total number of Anopheles mosquitos
cought between Jambi and Sumba (P value= <0.0001). Jambi
had mosquito abundance of 71 and Sumba had 2,364. Four
Anopheles species were successfully collected in Jambi, namely
Anopheles balabacensis, Anopheles barbirostris, Anopheles mac-
ulatus and Anopheles sinensis. An. balabacensis, which belongs
to leucosphyrus group, had the highest abundance, as shown
with its relative abundance of 78.87 and HLR of 0.52 per per-
son per night, followed by An. maculatus (relative abundance:
18.31 and HLR: 0.12 per person per night), An. barbirostris
(relative abundance: 1.41 and HLR: 0.01 per person per night)
and An. sinensis (relative abundance: 1.41 and HLR: 0.01 per
person per night). In contrast, the dominant Anopheles species
in Sumba were Anopheles aconitus and Anopheles sundacius,
with a relative abundance of 40.02 and 58.50 and HLR of 8.76
and 12.81 per person per night, respectively. The other minor
species found were An. barbirostris (relative abundance: 0.09
and HLR: 0.02), Anopheles farauti (relative abundance: 0.04
and HLR: 0.01), Anopheles leucosphyrus (relative abundance:
0.04 and HLR: 0.01), An. maculatus (relative abundance: 1.06
and HLR: 0.23), Anopheles subpictus (relative abundance: 0.17
and HLR: 0.04) and Anopheles vagus (relative abundance: 0.09
and HLR: 0.02) (Table 2).

There was a difference in Anopheles biting time between Jambi
and Sumba (Figure 1 and Figure 2). An. balabacensis from Jambi
has a peak in biting time during early evening (6 pm), which
decreases substantially until midnight, while An. maculatus
showed an irregular biting time pattern. On the other hand,
there is a similar trend in biting time between An. aconitus and
An. sundacius collected from Sumba; it gradually increased
until its peak biting time between 21.00-22.00 and 01.00-02.00;
then, it decreased progressively until 05.00-06.00. Additionally,
an irregular biting time pattern has also been observed for
An. maculatus from Sumba.

To investigate the biting preference of Anopheles mosquito, an
indoor and outdoor comparison was carried out (Figure 3 and
Figure 4). There was a statistically significant finding for the bit-
ing preference of An. balabacensis from Jambi; the number of
collected mosquitos from outdoor was higher than that of
Table 2. Species, total numbers of mosquitos collected, relative abundance and human landing rate of Anopheles mosquitoes from Jambi and Sumba.

<table>
<thead>
<tr>
<th></th>
<th>Jambi</th>
<th>Sumba</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Total collection</td>
</tr>
<tr>
<td>Jambi</td>
<td>An. balabacensis</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>An. barbirostris</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>An. maculatus</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>An. sinensis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>71</td>
</tr>
<tr>
<td>Sumba</td>
<td>An. aconitus</td>
<td>946</td>
</tr>
<tr>
<td></td>
<td>An. barbirostris</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>An. farauti</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>An. leucosphyrus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>An. maculatus</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>An. subpictus</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>An. sundicus</td>
<td>1,383</td>
</tr>
<tr>
<td></td>
<td>An. vagus</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2,364</td>
</tr>
</tbody>
</table>

Figure 1. Biting time pattern of Anopheles balabacensis and An. maculatus collected from Jambi. HLC, human landing catch.

indoor collection. No statistical difference was observed for An. maculatus. A similar pattern was found for An. aconitus, An. maculatus and An. Sundicus, where there was no difference between indoor and outdoor collection, suggesting that undertaking an indoor or outdoor activity carries the same risk of getting mosquito bites.

To investigate the difference in mosquito biting times between Jambi and Sumba, a multiple comparison analysis of pooled mosquito sample data was carried out (Table 3 and Figure 5). Based on the mosquito biting time in Jambi, the number of bites during the early evening (18.00-19.00) was statistically different from other biting times, from 21.00-22.00 to 05.00-06.00
Figure 2. Anopheles aconitus, An. maculatus and An. sundaicus biting times in Sumba. HLC, human landing catch.

Figure 3. Indoor and outdoor biting preference of Anopheles balabacensis (left) and An. maculatus (right) in Jambi. HLC, human landing catch.

Figure 4. Mean number of Anopheles aconitus (left), An. maculatus (center) and An. sundaicus (right) indoors and outdoors in Sumba. HLC, human landing catch.
Table 3. Summary of significant multiple comparisons between different mosquito biting times in Jambi and Sumba.

<table>
<thead>
<tr>
<th>Jambi</th>
<th>Dunn’s multiple comparisons test</th>
<th>Mean rank difference</th>
<th>Summary</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean rank difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 21.00-22.00</td>
<td>77.81</td>
<td>****</td>
<td>&lt;0.0001</td>
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<tr>
<td>18.00-19.00 vs. 22.00-23.00</td>
<td>67.15</td>
<td>***</td>
<td>0.0004</td>
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<tr>
<td>18.00-19.00 vs. 23.00-24.00</td>
<td>83.48</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 24.00-01.00</td>
<td>89.85</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 01.00-02.00</td>
<td>84.17</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 02.00-03.00</td>
<td>83.48</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 03.00-04.00</td>
<td>84.17</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 04.00-05.00</td>
<td>84.17</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 05.00-06.00</td>
<td>89.85</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>19.00-20.00 vs. 24.00-01.00</td>
<td>50.52</td>
<td>*</td>
<td>0.0435</td>
<td></td>
</tr>
<tr>
<td>19.00-20.00 vs. 05.00-06.00</td>
<td>50.52</td>
<td>*</td>
<td>0.0435</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sumba</th>
<th>Dunn’s multiple comparisons test</th>
<th>Mean rank difference</th>
<th>Summary</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean rank difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.00-19.00 vs. 20.00-21.00</td>
<td>-195.5</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>18.00-19.00 vs. 21.00-22.00</td>
<td>-238.1</td>
<td>****</td>
<td>&lt;0.0001</td>
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<td>18.00-19.00 vs. 22.00-23.00</td>
<td>-238.3</td>
<td>****</td>
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<td>18.00-19.00 vs. 23.00-24.00</td>
<td>-232.0</td>
<td>****</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>18.00-19.00 vs. 24.00-01.00</td>
<td>-214.9</td>
<td>****</td>
<td>&lt;0.0001</td>
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<tr>
<td>18.00-19.00 vs. 01.00-02.00</td>
<td>-204.8</td>
<td>****</td>
<td>&lt;0.0001</td>
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<tr>
<td>18.00-19.00 vs. 02.00-03.00</td>
<td>-180.1</td>
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<td>18.00-19.00 vs. 03.00-04.00</td>
<td>-147.6</td>
<td>**</td>
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<tr>
<td>19.00-20.00 vs. 21.00-22.00</td>
<td>-145.1</td>
<td>**</td>
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<td>19.00-20.00 vs. 22.00-23.00</td>
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<td>19.00-20.00 vs. 23.00-24.00</td>
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<td>21.00-22.00 vs. 04.00-05.00</td>
<td>150.2</td>
<td>**</td>
<td>0.0052</td>
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<tr>
<td>21.00-22.00 vs. 05.00-06.00</td>
<td>155.0</td>
<td>**</td>
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<tr>
<td>22.00-23.00 vs. 04.00-05.00</td>
<td>150.5</td>
<td>**</td>
<td>0.0050</td>
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<tr>
<td>22.00-23.00 vs. 05.00-06.00</td>
<td>155.2</td>
<td>**</td>
<td>0.0030</td>
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</tr>
<tr>
<td>23.00-24.00 vs. 04.00-05.00</td>
<td>144.2</td>
<td>**</td>
<td>0.0099</td>
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</tr>
<tr>
<td>23.00-24.00 vs. 05.00-06.00</td>
<td>149.0</td>
<td>**</td>
<td>0.0059</td>
<td></td>
</tr>
<tr>
<td>24.00-01.00 vs. 05.00-06.00</td>
<td>131.9</td>
<td>*</td>
<td>0.0347</td>
<td></td>
</tr>
</tbody>
</table>

* <0.05, ** <0.01, *** <0.001, **** <0.0001.

(P value= <0.00001). Additionally, the number of bites at 19.00-20.00 was statistically different from the number at 24.00-01.00 and 05.00-06.00 (P value= 0.0435). In Sumba, the number of bites during the early evening at 18.00-19.00 was statistically different from the other biting times, except for 19.00-20.00, 04.00-05.00 and 05.00-06.00 (P value= <0.0001-0.0069). In addition, the number of bites at 19.00-20.00 differed from the number at 21.00-22.00, 22.00-23.00 and 23.00-24.00 (P value= 0.0088-0.0168); 21.00-22.00 differed from 04.00-05.00 and 05.00-06.00 (P value= 0.0030-0.0052); 22.00-23.00 differed from 04.00-05.00 and 05.00-06.00 (P value= 0.0030-0.0050); 23.00-24.00 differed from 04.00-05.00 and 05.00-06.00 (P value= 0.0059-0.0099); and 24.00-01.00 differed from 05.00-06.00 (P value= 0.0347). These results indicate that in Jambi,
the peak biting time is during early evening at 18.00-20.00. In Sumba, the mosquitoes started feeding and feeding gradually intensified during the early evening (18.00-21.00), the intensity of the mosquitoes was stable until 02.00 and then the mosquito biting intensity declined during the early morning.

Discussion

According to the Malaria Atlas Project\textsuperscript{30}, for API <0.1, \textit{Plasmodium falciparum} and \textit{Plasmodium vivax} distributions are similar across the Indonesian archipelago. \textit{Plasmodium falciparum} is more stable in distribution, where each part of Indonesian archipelago has the same pattern of low to moderate API. Meanwhile, \textit{Plasmodium vivax} is more intense in the eastern part of Indonesia and unstably distributed in the western part of Indonesia. However, only \textit{Plasmodium vivax} was found in Jambi, and more diverse \textit{Plasmodium} species have been observed in Sumba, suggesting a different diversity of \textit{Plasmodium} species distribution in the two localities. A discrepancy was also found in the calculated API between this study and the basic health report by the Ministry of Health of Indonesia, which might be explained by the different ways of presenting the data. The national health report\textsuperscript{23} used the provincial population and the larger the area, the larger the population involved in the calculation, as API is calculated by dividing the total cases and the total population. API at a sub-district level is often observed to vary from one district to another and variation between districts is observed at a provincial level\textsuperscript{31,32}.

There are 20 \textit{Anopheles} species known to be vectors for malaria in Indonesia. In this study, four and eight species have been found in Jambi and Sumba, respectively. The student t-test suggested a different abundance in the number of \textit{Anopheles} mosquitoes between the two sites. This difference is often explained by environmental conditions. A distinct sampling time may cause this difference in mosquito abundance; however, since rainfall anomalies have been observed in Indonesia, this may not be the case\textsuperscript{33}. Since the existence of \textit{Anopheles} breeding sites depends on rainfall providing a sufficient water bodies for the mosquitoes to lay eggs, rainfall anomalies in Indonesia may lead to to an irregular pattern of mosquito abundance across time and place in Indonesia. The limited number of water bodies or humidity conditions may affect the habitat and abundance of \textit{Anopheles} mosquitoes in Jambi\textsuperscript{34,35}. The difference in mosquito abundance may also reflect the annual incidence rate of malaria infection in different endemic areas. However, no correlation may be found if the correlation of mosquito abundance and annual incidence rate takes into account the species of \textit{Plasmodium}\textsuperscript{36}.

The main \textit{Anopheles} vector and biting preference differs between Jambi and Sumba. \textit{An. balabencis}, which belongs to leucosphyrus group, is the primary vector in Jambi, as determined from its highest relative abundance and HLR. Moreover, \textit{An. aconitus} and \textit{An. sundaicus} are the primary vectors in Sumba, along with other minor \textit{Anopheles} species found. Only \textit{An. balabacensis} in Jambi was found to be exophagic, as previously known from the biting preference of this peculiar species\textsuperscript{37}. \textit{An. maculatus} has been found to be both endophagic or exophagic similar to the findings of Elyazar \textit{et al.}\textsuperscript{37}. However, previous studies have found that \textit{An. aconitus} has an irregular pattern of biting preference while \textit{An. sundaicus} is mainly exophagic\textsuperscript{37}. This study found that there was no significant difference between the indoor and outdoor biting preference of \textit{An. aconitus} and \textit{An. sundaicus}, suggesting that these species can be both endophagic and exophagic.

Biting time is essential to understanding the underlying biological properties of mosquitoes and to avoid \textit{Anopheles} bites to control malaria infection. The data obtained suggest different biting times of \textit{Anopheles} in Jambi and Sumba. Early evening (18.00-20.00) is most likely to be the mosquito feeding time in Jambi, when most people are undertaking activities and

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mosquito_biting_time.png}
\caption{Mean number of \textit{Anopheles} mosquito at different biting times in Jambi (left) and Sumba (right). HLC, human landing catch.}
\end{figure}
are unprotected. However, in the late evening (21.00-02.00), more people in Sumba may get Anopheles bites, reflecting sleeping time, when Sumbanese people may be vulnerable to infection with malaria parasites. This suggests the importance of ITNs for evading malaria infection in Sumba. The biting time of Anopheles in Jambi is similar to that in Halmahera, Maluku Island\(^2\). However, the finding from Sumba Island is different from other parts of Indonesia, which shows a gradual increase or decrease in the number of Anopholes mosquitoes in accordance with its biting time\(^2\). Limited studies have tried to describe mosquito biting patterns in relation to the selection of malaria control strategies\(^2,21\). This finding strengthens the previous report that effective malaria prevention depends on local Anopheles vector biting behavior. Anopheles vectors in Jambi share the same behavior as those in Burkina Faso, where bed net protection may not be effective for preventing biting exposure as Anopheles species in the area are dominant in the early evening\(^21\). In contrast, similar to Uganda, intensive use of ITNs combined with indoor residual spraying is the most effective protection approach for Sumba Island for avoiding malaria infection\(^20\).

Biting preference has previously been known to have an underlying genetic background\(^1\). For instance, chromosome inversions of 2Rbc, 2Ra and 3Ra and circadian clock genes are associated with exophagic and endophagic behavior in some Anopheles species\(^3,4\). However, genetic background may vary within the genus and among mosquitoes within the same species in different locations\(^4\). The finding also suggests that differences in Anopheles biting time may be an effect of different genetic backgrounds. Further research might explore this aspect.

There are some limitations of the current study. There was no intervention included to measure the effectiveness of any type of protection in correlation with the different biting times in each study site. In further research, an intervention approach should be used to find the best protection strategy in locations that may have different Anopheles biting times. Additionally, our collection method was limited to three weeks observational research. A more prolonged study needs to be conducted to reflect yearly fluctuations in local Anopheles biting times.

**Conclusion**

In conclusion, this study suggests four important findings for public health control: (1) API may be significantly lower at the provincial level compared to the sub-district level and varied accordingly, suggesting that malaria foci may be maintained in a locality from a provincial level, especially in areas of low to moderate endemicity; (2) the importance of mosquito abundance information may reflect malaria incidence rate in a location\(^2,23\); (3) all Anopheles species, except An. balabacensis, can be both endophagic and exophagic, suggesting a comprehensive protection approach is required to avoid mosquito bites regardless of being indoors or outdoors; (4) biting time may suggest the use a different prevention approach in each area; for example, people in Jambi may need to use mosquito repellent during activities in the early evening, while ITNs combined with indoor residual spraying may need to be deployed to protect malaria infection during sleeping hours in Sumba.

**Data availability**

**Underlying data**

Zenodo: The diversity of Anopheles blood feeding patterns suggest different malaria protection strategies in different localities. [https://doi.org/10.5281/zenodo.3269824\(^29\)]

This project contains the following underlying data:

- Supplementary 1.xls (The total number of mosquitoes collected, number collected per time period and number collected indoors/outdoors)
- Supplementary 2.xls (The number of mosquitoes caught for each species in Jambi and relative abundance and HLC calculations)
- Supplementary 3.xls (The number of mosquitoes caught for each species in Sumba and relative abundance and HLC calculations)
- Supplementary 4.xls (Results of all Dunn’s multiple comparisons tests for biting times in Jambi and Sumba)
- Supplementary 5.docx (Flow chart of the HLC collection method)
- Supplementary 6.rar (detailed data of all Anopheles found in Jambi per collection type and collection time)
- Supplementary 7.zip (detailed data of all Anopheles found in Sumba per collection type and collection time)
- Supplementary 8.xlsx (demographic data and parasite species for participants from both study sites)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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