Research Article

Local Diversity and Biting Pattern of Anopheles Species in Southern Minahasa

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Background. To optimize the preventive measures of malaria, it is important to synchronize the efforts with the behavior of local Anopheles species. However, the data of Anopheles species and their behavior in Indonesia is still lacking.

Method. Explorative research was conducted from April to September 2016 in Southern Minahasa district. The Anopheles mosquito was baited by using animal and human (indoor or outdoor) from 18.00 to 06.00 hours. Then, the species were identified and Man Biting Rate (MBR) and Man/Animal Biting per Hour (MBPH) were calculated followed by statistical analysis by using SPSS 17.

Result. The data showed that the dominant species in Southern Minahasawere An.barbirostris, An.kochi, and An.vagus. An.vagus was found to be zoophilic and An. barbirostris was showing strict anthropophilic characteristics. Meanwhile, An. kochi feeds on both human and animal. The MBR of An. kochi was found to be the highest (P < 0.005), but its MBPH only significantly exceeded that of An. vagus. All species tend to be more active during the early evening.

Conclusion. An.barbirostris, An.kochi, and An.vagus were the dominant Anopheles species in Southern Minahasa. Further research is needed to determine the Plasmodium infestation rate of these species.

1. Background

The mosquito is known as a vector for some of the most important human parasites including malaria which is one of important communicable diseases especially in tropical area [1]. Global prevalence rate of malaria was estimated at 214 million cases with 438,000 deaths in 2015 [2]. It is also one of the main burdens of infectious disease in Indonesia with 209,413 new cases in 2015. It is particularly concentrated in Middle and Eastern Indonesia especially in Southern Minahasa which experienced a rise in malarial cases [3].

The diversity of Anopheles mosquito poses a real challenge to malaria control programs because different species tend to have different behaviors and feeding locations (indoor versus outdoor) [4]. For example, if predominant Anopheles species within the region tend to feed on human outdoor, then common preventive measures like insecticide-impregnated bed nets and indoor spraying will be useless to prevent malaria. Also, the zoophilic tendency is also important in the case of zoophilic-anthropophilic Anopheles since the presence of domestic animal near housing will only attract mosquito [5]. Furthermore, this type of Anopheles also increases the risk of zoonosis, such as in the case of P.knowlesi [6].

Since there are only few studies of Anopheles species in Indonesia, the exploration and mapping of Anopheles species and its distribution are important. Moreover, Indonesia is an archipelago country with each of its islands or regions having very distinct ecological characteristics from one another; thus it could be hypothesized that the Anopheles diversity will also differ from one area to another. So this type of research is urgently needed in Indonesia, especially in malaria endemic areas such as Southern Minahasa.
2. Methods

A descriptive explorative research was conducted to identify *Anopheles* species in the region of South Minahasa district and evaluate whether the species are potential vector for malaria from April to September 2016 in South Minahasa district. Sampling was conducted in the areas suspected as *Anopheles* breeding ground for two weeks. Mosquitoes were baited using human and animal and then caught using insect net. The mosquitoes were then placed inside a labeled tube film, identified, and counted. The protocol of using human and animal bait is described as follows.

2.1. Human Bait. The mosquitoes were lured by using four people as human baits who worked in shifts between 18.00 and 06.00 hours. As a preventive measure, malaria prophylaxis (chloroquine) had been given one week before research and continued for up to 1 month after the end of the study. Sample collection was conducted both inside and outside the house from families that had at least one member with malaria. The human bait wore a short and rolled up the cloth sleeve, exposing the whole arm to the mosquito. The human bait was also asked not to smoke during the baiting period.

2.2. Animal Bait. Mosquito catching using animal baits was conducted in the animal pen and its surrounding areas with no special treatment given to the animal. All mosquitoes that landed on the animal were collected using aspirator and stored inside labeled container.

All collected specimens were identified using taxonomical book by O'Connor and Soepanto (1999) [7].

The density of mosquitoes that contact the human and animal bait was calculated by using following equation.

(a) The Man Biting Rate (Man Biting Rate) [8]:

\[
\text{MBR} = \frac{\text{the number of mosquitoes on human bait per night}}{\text{the number of catching attempts} \times \text{period of catching activity}}. \tag{1}
\]

(b) Man/Animal Biting per Hour (MBPH) [9]:

\[
\text{MBPH} = \frac{\text{the number of mosquitoes on human bait per hour}}{\text{the number of catching attempts} \times \text{period of catching activity}}. \tag{2}
\]

All data were analyzed descriptively to see the diversity of the species, the pattern of mosquito density, and its biting pattern. All data were analyzed by normality test (Shapiro-Wilk) to determine the distribution pattern. Next, we compared the density and biting pattern of each species to evaluate whether the differences were statistically significant. All analyses were conducted with SPSS17 for Windows.

3. Results

3.1. Diversity Profile of Anopheles Mosquito Caught in Southern Minahasa. Table 1 describes the profile of *Anopheles* species that was found in Southern Minahasa as well as their respective frequency from May to September 2016.

Table 1: Profile of *Anopheles* mosquito species and the average number of each species with human and animal baits.

| Period of catch | Indoor Human bait (1) | Indoor Animal bait (2) | Outdoor Human bait (3) | Outdoor Animal bait (4) | Animal bait (5) |
|-----------------|-----------------------|-----------------------|-----------------------|------------------------|----------------|
| May             | 17                    | 4                     | 17                    | 9                      | 14             |
| June            | 19                    | 17                    | 7                     | 7                      | 26             |
| July            | 20                    | 29                    | 20                    | 3                      | 28             |
| August          | 27                    | 30                    | 5                     | 4                      | 39             |
| September       | 32                    | 14                    | 4                     | 5                      | 32             |

Average of MBR ranges from 0.71 to 0.89 inside the house and 0.02 to 0.35 outside the house. Meanwhile, MBPH of this species ranged from 0.3 to 0.9 mosquitoes per animal. Meanwhile, evaluation of MBPH revealed that the feeding activity of *An. vagus* took place between 18.00 and 22.00 hours with a momentary pause between 22.00 and 05.00 hours. Then, the feeding activity resumed briefly from 05.00 to 06.00 hours. The MBPH of this species ranged from 0.1 to 0.2 mosquitoes/animal/hour.

3.2. The Density and Biting Pattern of Anopheles Mosquito in Southern Minahasa. Next, we calculated the MBR of *An. vagus*, *An. kochi*, and *An. barbirostris*. The result of MBR of those species is described in detail in Tables 2, 3, and 4. Because *An. vagus* feeds on animals, we calculate its MBR and MBPH based on the number of mosquitoes found feeding on domestic animals. Table 2 shows that the MBR of this species ranges from 0.3 to 0.9 mosquitoes per animal. Meanwhile, *An. kochi* was found in human bait experiment, but both of them were commonly found in animal bait experiment.

We evaluated the same variable for *An. kochi*. It appears that the MBR of *An. kochi* in animals ranges from 0.31 to 1.64, which was higher than that of *An. vagus*. Also, its MBPH ranged from 0.02 to 0.35 inside the house and 0.02 to 0.54 outside the house. MBPH data indicate that the feeding activity of *An. kochi* was found in human bait experiment, but both of them were commonly found in animal bait experiment.
### Table 2: Man Biting Rate (MBR) of *An. vagus*, *An. kochi*, and *An. barbirostris*.

| Month       | *An. vagus* | *An. kochi* | *An. barbirostris* |
|-------------|-------------|-------------|-------------------|
|             | Animalbait  | Animalbait  | Exophytichumanbait |
| May         | 0.7         | 0.7         | 0.45              |
| June        | 0.9         | 0.83        | 0.29              |
| July        | 0.5         | 1.64        | 0.11              |
| August      | 0.3         | 0.59        | 0.13              |
| September   | 0.6         | 0.31        | 0.14              |

### Table 3: Man/Animal Biting per Hour (MBPH) of *An. vagus*, *An. kochi*, and *An. barbirostris*.

| Timing period | *An. vagus* | *An. kochi* | *An. barbirostris* |
|---------------|-------------|-------------|-------------------|
|               | Exophytic humanbait | Endophytichumanbait | Exophytic humanbait | Endophytichumanbait |
| 18.00–19.00   | 0.1         | 0           | 0.29              |
| 19.00–20.00   | 0.2         | 0           | 0.46              |
| 20.00–21.00   | 0.2         | 0           | 0.54              |
| 21.00–22.00   | 0.1         | 0           | 0.39              |
| 22.00–23.00   | 0           | 0           | 0.39              |
| 23.00–24.00   | 0           | 0           | 0.33              |
| 00.00–01.00   | 0           | 0           | 0.13              |
| 01.00–02.00   | 0           | 0           | 0.02              |
| 02.00–03.00   | 0           | 0           | 0.02              |
| 03.00–04.00   | 0           | 0           | 0.02              |
| 04.00–05.00   | 0           | 0           | 0.02              |
| 05.00–06.00   | 0.1         | 0           | 0.02              |

3.3. **Comparison of MBR and MBPH between *An. vagus*, *An. kochi*, and *An. barbirostris*.** Lastly, we compared both variables (MBR and MBPH) between *An. vagus*, *An. kochi*, and *An. barbirostris*. The details of comparisons are presented in Tables 4 and 5. From MBR perspective, it was clear that the exophytic density of *An. vagus* and *An. kochi* exceeded that of *An. barbirostris* significantly. In addition, the endophytic density of *An. barbirostris* also exceeded its exophytic density. Meanwhile, from MBPH data, it was clearly seen that only the difference between MBPH of *An. vagus* and exophytic *An. kochi* was statistically significant (Table 5). Considering that all species tend to be more active during the first half of the night, we decided to exclude the other 6-hour periods of observation from analysis (reanalysis; Table 5). On reanalysis, it appeared that the endophytic and exophytic activity of *An. kochi* were significantly different. Meanwhile, no significant difference was observed between exophytic and endophytic *An. barbirostris*.

### Table 4: MBR comparison between *An. vagus*, *An. kochi*, and *An. barbirostris*.

| Species | Species comparison | *P* value |
|---------|--------------------|-----------|
| *An. vagus* | Endophytic *An. barbirostris* | 0.073     |
| *An. vagus* | Exophytic *An. barbirostris* | 0.014     |
| *An. kochi* | Endophytic *An. barbirostris* | 0.251     |
| *An. kochi* | Exophytic *An. barbirostris* | 0.016     |

### 4. Discussion

Malaria is one of the most deadly and widespread parasitic disease in the world [2]. Many attempts had been made to control, prevent, and even eliminate the disease. One of them is by studying the species diversity and behavior of *Anopheles* mosquito, the vector of malaria [4]. This field of research is considered to be important because it could identify the specific species of *Anopheles* responsible for malaria transmission from human to human or animal to human and vice versa [6]. It could also contribute to malaria prevention by matching the preventive programs to predominant *Anopheles* species within the area [4].
There are several interesting facts that we obtained from this exploratory study. We found there were five species of Anopheles in the Southern Minahasa region: An. barbirostris, An. parangensis, An. maculatus, An. vagus, and An. kochi. The first three species were found to feed on humans; meanwhile, the other two were found to only feed on animals. Meanwhile, An. kochi was found to feed on both humans and animals. The frequency of each species is shown in Table 1.

Our study results clearly showed that An. barbirostris and An. kochi were the predominant species. This finding is different from that reported in studies conducted in other locations in Southeast Asia, such as Malaysia, Thailand, Vietnam, and Lao PDR, where it was reported that An. maculatus is the dominant species [6,10–12]. Similarly, Sumba Island, which is also in the Central Indonesia region, is dominated by another species, namely, An. sondaicus [13]. However, our results are indeed consistent with studies conducted by Ndoen et al. and Pinontoanet al. (unpublished), but An. kochi was not considered to be a common species by both studies [14,15]. The reason for the discrepancy might be because the population of An. barbirostris, An. parangensis, and An. maculatus tends to be higher in populated areas, especially inside the house, which increases the chances of catching in this study [4,14]. Meanwhile, this study focused on the peripheral area which is less populated but close to shrub in this study [4,14]. Meanwhile, this study focused on the inside the house, which increases the chances of catching maculatus tends to be higher in populated areas, especially 0.025mosquitoes/human/night[16].However, MBR rates exceeded the threshold level for vector, which is lyzed species were considered potential vectors because their to the cattle pen [18].

By comparing their MBR and MBPH rates, all the analyzed species were considered potential vectors because their MBR rates exceeded the threshold level for vector, which is 0.025 mosquitoes/human/night [16]. However, An. vagus fed specifically only on domestic animals and showed no interest toward human. The preference of An. vagus for animals to humans was also reported by several other researches [5, 14, 17, 18]. However, it had also been identified as malaria vector in someoccasions,especiallyduringoutbreaks[19]. Furthermore, it was also found to bite people who live close to the cattle pen [18].

On the other hand, An. barbirostris was found to specifically feed on humans, which was confirmed by the absence of this species during the animal bait experiment. The density (MBR) of this species was higher than that of An. vagus but lower than that of An. kochi, though the difference between it and An. vagus was not statistically significant. It was also found both inside and outside the house, but its endophytic MBR was significantly higher, which suggests that this species was more active indoor. Based on the pattern of its MBPH, it was found that An. barbirostris tended to remain active during the first 4-5 hours of the evening. However, the MBPH pattern showed that this species was slightly more active outdoor. An. barbirostris is widely distributed in Southeast Asia and some studies had also confirmed it as a Plasmodium vector [20,21]. However An. barbirostris was never found to be a common species in other studies conducted in Southeast Asia and its density had always exceeded the density of An. maculatus, An. sondaicus, and An. dirus [6,10]. Furthermore, Amerasinghe et al. and Reid et al. reported that it is the anthropophilic-zoophilic type which is contrary to our findings [22, 23].

As for the comparison of the last two Anopheles species, the feeding pattern of An. kochi was found to be similar to that of An. barbirostris, but An. barbirostris was found feeding on both humans and animals. By comparing its MBR and MBPH, it was clear that the density of this species exceeded the densities of the other two species. Furthermore, its exophytic feeding activity was also higher than its endophytic activity, although there were no statistically significant differences between its MBPH and that of An. vagus and exophytic An. barbirostris. This species tends to be more widely distributed compared with other Anopheles species, which is confirmed by Pinontoan [15]. The same study had also stated that this species was invested with Plasmodium, which highlights the potential role of this species in malaria transmission. However, a study conducted in Maluku region of eastern Indonesia by Soekirno et al. reported that An. kochi was not infectious and did not act as an effective vector for Plasmodium [24]. Jiram et al. also studied this species to find out whether it was susceptible to animal Plasmodium infection but could not find any sporozoite [6]. Thus, it remains unclear whether this species could act effectively as a Plasmodium vector and further researches are needed to confirm the findings reported by Pinontoan [15].

Overall, our findings reveal the local diversity in Southern Minahasa region of Celebes Island, Indonesia. This finding was different compared with the other studies conducted in Southeast Asia. In this study, the density of An. maculatus was found to be lower than that of the other four species; meanwhile, it was found to be predominant in several countries of Indochina peninsula [4,10–12]. Furthermore, An. farauti and An. punctulatus are predominant in Papua New Guinea and An. sondaicus is commonly found in Sunda Island, Indonesia [13, 25, 26]. It seems there is great diversity
in species that contributes to malaria transmission between different areas in Southeast Asia. However, all studies stated that Anopheles mosquito tends to be more active during the first hour of the evening, which is in accordance with the findings of this study. Nevertheless, this study provided additional data about Anopheles species in Indonesia which still has high prevalence of malaria.

5. Conclusion

Based on the results of this study, we found that An. kochi and An. barbirostris were the main vectors of malaria in Southern Minahasa district, with An. barbirostris feeding specifically on humans and An. kochi feeding on both humans and animals. It also appears that they tend to be more active during the first hour of the evening whereas their activity drops significantly later on. In regard to An. kochi's feeding behavior, it can be concluded that having a domestic animal around the settlement could attract this species and, hence, increase the risk of malaria infection. However, further research is needed to investigate the Plasmodium infestation rate among these Anopheles species in order to complete the map of species distribution and susceptibility areas in Minahasa Region and Indonesia.

Conflicts of Interest

The authors certify that they have no affiliation with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interests; and expert testimony or patent-licensing arrangements) or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Authors’ Contributions

All authors contributed equally in the processing and writing of this research.

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