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Study of Behavior, Distribution, and Potential as a Vector of Malaria *Anopheles letifer* In Indonesia

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ABSTRACT

Background: Anopheles letifer is one of the malaria vectors in Indonesia. This species was previously known to be distributed in North Sumatra, Riau, Jambi, South Sumatra, West Kalimantan, Central Kalimantan, South Kalimantan, East Kalimantan and Bangka Belitung provinces. The potential of An. letifer as a malaria vector is influenced by its behavior. Based on this background, the study aims to determine the development of the distribution, and behavior of An. letifer and the potential transmission of malaria in Indonesia.
Method: Mosquito collection was carried out using human landing methods, animal bit traps, collection around livestock, resting morning, and light traps. The mosquitoes have analyzed the presence of Plasmodium, the blood feed analysis, and fluctuations in mosquito density throughout the night.
Results: The results showed that An.letifer was distributed on Riau, Riau Islands, Jambi, Bangka Belitung, West Kalimantan, Central Kalimantan, South Kalimantan and North Kalimantan provinces. Anopheles letifer was known to predominantly suck the blood of people outdoor and some indoors. This species has a night-long blood-sucking activity with varying densities and fluctuations throughout the night in each province. The results of the analysis of Plasmodium positive in An. letifer were found in Central Kalimantan.
Conclusion: The potential transmission of malaria transmitted by An. letifer was higher in Central Kalimantan when compared to other provinces.

1. Introduction

Anopheles letifer is one of the malaria vectors in Indonesia. Distribution An. letifer as a malaria vector is found in the provinces of North Sumatra, Riau, Jambi, South Sumatra, West Kalimantan, Central Kalimantan, South Kalimantan, East Kalimantan and in Bangka Belitung (1–3). Distribution An. letifer are also found in Sarawak and Hulu Selangor Malaysia (4),(5). *Anopheles letifer* as a vector occurs not in all regions in Indonesia. Mosquitoes can act as vectors because they have the gene arrangement in the salivary glands as well as the composition of the COI mtDNA and ITS2 genes in rDNA (6),(7). The potential of mosquitoes as vectors can be seen from their behavior.

Mosquitoes that have close proximity to humans have greater potential for malaria transmission. This can be seen from his behavior in sucking blood. Another factor that affects the ability of mosquitoes to act as vectors is resistance to pathogens that enter their bodies. Mosquitoes that act as malaria vectors have resistance to Plasmodium that enters their bodies so that they are able to survive and the pathogen cycle can take place before being transmitted to humans[8–10].

The study of vector behavior is very necessary in the context of efforts to reduce malaria cases. Knowing the behavior of vectors will help vector control efforts to be on target [11]

Studies of the behavior of *An. letifer* are not widely known even though this species is known to be a malaria vector in Indonesia. Previous studies in Batam found *An. letifer* mosquitoes both in the adult and larval stages with low populations. Larva *An. letifers* are found in swamps and mangrove swamps 2. Remembering *An. letifer* has been proven to be a malaria vector in several provinces in Indonesia, so the behavioral study of *An. letifer* needs to be carried out to detect the potential for malaria transmission transmitted by *An. letifer*. Based on this background, this study aims to obtain information on the distribution and behavior of *An. letifer* as well as the potential for malaria transmission in several provinces in Indonesia.

2. Method

Write This research was conducted in 29 provinces in Indonesia. Each province was taken by three districts. Each district took six sampling points. The sampling points in each district represent three ecosystems, namely forest, non-forest and coastal ecosystems. Meanwhile, each ecosystem was taken from two points, namely the near and far ecosystems of thought. So that the total sampling points in each district were six points, there are forest near settlements (FNS), forest far settlements , (FFS) non forest near settlements (NFNS), non forest far settlements (NFFS), beach near settlements (BNS), and beach far settlemets (BFS). The list of sampling locations can be seen in Table 1 Sampling at each point is carried out for five days, so that in one district sampling was carried out for 30 days.

Table 12. Mosquito sampling locations in several provinces in Indonesia

No	Provinsi	Nama Kota/ Kabupaten
1	Papua	Biak, Meraoke, Sarmi
2	Central Java	Pati, Pekalongan, Purworejo
3	South Sumatera	Banyuasin, Lahat, Ogan Komering Ilir (OKI)
4	South Sulawesi	Parigi Mautong, Tojo Una Una
5	Aceh	East Aceh, West Aceh, Pidie
6	West Sumatera	South Pesisir, Padang Pariaman, West Pasaman
7	Lampung	Tanggamus, South Lampung Pasawaran
8	Bangka Belitung	Bangka, Belitung, Central Bangka
9	West Java	Garut, Subang, Pangandaran
10	East Java	Malang, Banyuwangi, Pasuruan
11	Banten	Pandeglang, Lebak, Serang
12	Nusa Tenggara Barat	West Lombok , Bima, north Lombok
13	Nusa Tenggara Timur	Belu, Ende, Central Sumba
14	West Kalimantan	Sambas, Ketapang, North Kayong
15	South Kalimantan	Barito Kuala, Kota Baru, Tanah Laut
16	Nort Sulawesi	Minahasa, Kota Manado, Kota Bitung
17	South east Sulawesi	Muna, Konawe, Bombana
18	Maluku	West Southeast Maluku, southeast Maluku, Kepulauan Aru
19	North Maluku	Central Halmahera, South Halmahera, Pulau Morotai
20	Riau	Bengkalis, Dumai, Kepulauan Meranti
21	Jambi	Bungo, Sarolangon, Tanjung Jabung Barat

22	DIY	Kulon Progo, Gunung Kidul, Bantul
23	Bali	Jembrana, Badung, Karangasem
24	Central Kalimantan	Gunungmas, Pulangpsau, Murung Raya
25	South Sulawesi	Pangkep, Bulukumba, East Luwu Timur
26	West Papua	Raja Ampat, manokwari, Fak fak
27	Kepulauan Riau	Kota Batam, Bintan, Lingga
28	Dki Jakarta	Central Jakarta, West Jakarta, South Jakarta, East Jakarta, Kepulauan Seribu
29	North Kalimantan	Bulungan, Nunukan, Tarakan

The sampling process was carried out by catching mosquitoes using several methods including human bait (indoor and outdoor), animal bited traps (ABT), catching around livestock, light traps, and catching mosquitoes in the morning (Resting morning).

Mosquito catching activities take different times according to the method used. Mosquito catching by human bait method, ABT, catching around livestock and light traps was carried out at 18.00-06.00. The time to catch mosquitoes using the human bait method was 40 minutes per hour, while the ABT method and surveys around the livestock pen were 15 minutes. In the light trap method, the installation of traps was carried out from the afternoon and the collection of caught mosquitoes was carried out the next morning. Meanwhile, the method of catching mosquitoes in the morning/resting morning was carried out at 06.00-09.00 in places that have the potential to be a mosquito rest, such as earthen cliffs and stones, plant roots, and others.

Mosquitoes caught at various methods and times of capture were identified by their species using a mosquito identification key (12),(13). Anopheles mosquitoes that have been identified have their thorax heads cut off and then analyzed for the presence of Plasmodium using PCR in the laboratory. The primers used to detect the presence of Plasmodium are rPLU1: 5'-TCA AAG ATT AAG CCA TGC AAG TGA-3'; rPLU5 :5'-CCT GTT GTT GCC TTA AAC TCC-3' followed by Nest 2 genus-specific primers rPLU 3: 5'-TTT TTA TAA GGA TAA CTA CGG AAA AGC TGT-3'; rPLU 4 : 5' – TAC CCG TCATAGCCA TGT TAG GCC AAT ACC-3 (14), (15).

Mosquitoes An. letifer that are caught containing blood or half gravid will be tested using PCR. The mosquitoes used for this examination are mosquitoes from the results of the capture using the resting morning and light trap methods. The purpose of this blood test was to find out the preference for blood, whether human or animal blood (14), (15).

3. Result

Based on the results of mosquito catching in 29 provinces in Indonesia, there are 8 provinces where *An letifer* was positive for Plasmodium. The total number of mosquitoes caught were 3,235 tail. The results of the study show that An. letifer in Indonesia were distributed in Riau Province, Riau Islands, Jambi, Bangka Belitung, West Kalimantan, Central Kalimantan, South Kalimantan and North Kalimantan. The distribution of these species can be seen in Figure 1.

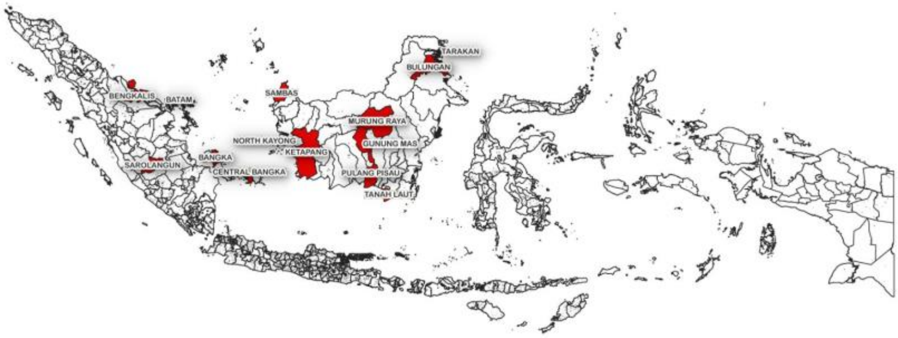

Figure 5. Map of the distribution of An.letifer locations in Indonesia

The results of the mosquito catch showed that the fluctuations and density of the *An. letifer* different in each province. Jambi Province was a province with a density of *An. letifer* was the highest compared to other provinces. Some provinces with high density of *An. letifer* under Jambi Province include Bangka Belitung Province and West Kalimantan. The fluctuations in mosquito density in each province can be seen in Figure 2. The number of *An. letifer* mosquitoes caught by various capture methods can be seen in Table 2.

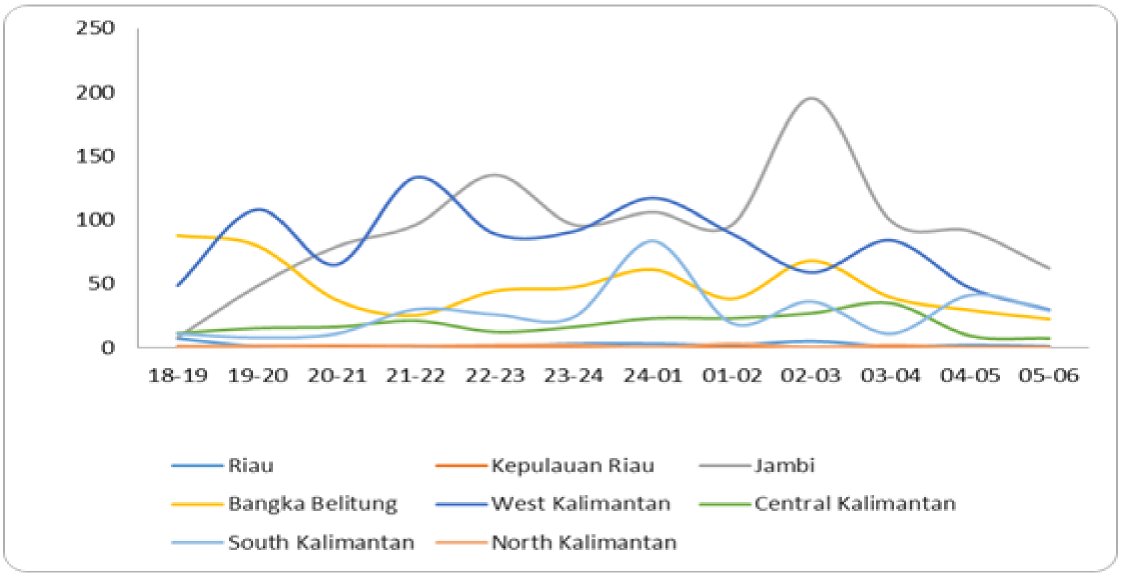

Figure 6. Fluctuations in the density of mosquitoes An. letifer in several provinces in Indonesia.

The results of the overall mosquito catch show that *An. letifer* found 96.69% suck human blood, with the vast majority (93.63%) sucking human blood outdoors. *Anopheles letifer* that sucks livestock blood using both livestock feed and ABT methods is only 3.31%. Description of blood-sucking behavior *An. letifer* in each province can be seen in Table 3. Based on the analysis of blood feed from the morning mosquito catch, *An. letifer* tend to suck human blood. *Anopheles letifer* which is known to suck human blood was found in Bangka Belitung and Central Kalimantan Provinces (Table 3). The results of the malaria examination showed that *An. letifer* that were caught positive for Plasmodium were found in Central Kalimantan Province (Table 4).

Table 13. The number of mosquitoes An. letifer are caught by various fishing methods in several provinces in Indonesia

Number	Province	Number of mosquitoes by method				
		ABT	*LF*	*IB*	*OB*	*RM*
1	Riau	0	0	0	26	0
2	Kepulauan Riau	0	0	0	3	0
3	Jambi	60	10	9	1032	0
4	Bangka Belitung	0	0	68	510	0
5	West Kalimantan	21	0	0	940	0
6	Central Kalimantan	2	4	21	188	0
7	South Kalimantan	0	7	1	321	0
8	North Kalimantan	3	0	0	9	0
Total number of mosquitoes		86	21	99	3029	0

Keterangan;
ABT : Animal bited trap
LF : livestock feed
IB : Indoor bait
OB : Outdoor bait
RM : Resting morning

Table 14. The results of the blood feed examination of An. letifers in several provinces in Indonesia

Number	Province	Blood feed analysis	
		Blood of human	*Blood of animal*
1	Riau	-	-
2	Kepulauan Riau	-	-
3	Jambi	-	-
4	Bangka Belitung	+	-
5	West Kalimantan	-	-
6	Central Kalimantan	++	-
7	South Kalimantan	-	-
8	North Kalimantan	-	-

Information:
+ Positive
- Negative

Table 15. The result of analysis Plasmodium An. letifer in some province in Indonesia

No	Provinsi	Metode penangkapan				
		ABT	*LF*	*IB*	*OB*	*RM*
1	Riau	-	-	-	-	-
2	Kepulauan Riau	-	-	-	-	-
3	Jambi	-	-	-	-	-
4	Bangka Belitung	-	-	-	-	-
5	West Kalimantan	-	-	-	-	-
6	Central Kalimantan	-	-	-	-	+
7	Kalimantan Selatan	-	-	-	-	-
8	North Utara	-	-	-	-	-

Keterangana;
ABT : *Animal bited trap*
LF : livestock feed
IB : Indoor bait
OB : Outdoor bait
RM : *Resting morning*
+ : Positive *Plasmodium*
- : Negative *Plasmodium*

4. Discussion

Based on the results of the study, it shows that of the 29 provinces surveyed by *An. letifer* was known to be found only in 8 provinces, there were Riau Province, Bangka Belitung, South Kalimantan, Riau Islands, West Kalimantan, North Kalimantan, Jambi, and Central Kalimantan. This shows that there was an addition to the distribution area of *An. letifer*. The results of previous data in Indonesia *An. letifer* was known to be a malaria vector in North Sumatra, Riau, Jambi, South Sumatra, West Kalimantan, Central Kalimantan, South Kalimantan, and East Kalimantan. With the increase in the distribution area of *An. letifer*, will affect the potential spread of malaria transmitted by this species 1. *Anopheles letifer* was also known to be distributed in Sarawak, Malaysia and Singapore (16),(17).

Potential *An. letifer* as a malaria vector can be shown from the results of confirmed positive examination of *Plasmodium* in Central Kalimantan province. This shows the potential for an increase in malaria cases in the region. This result is in line with several districts in Central Kalimantan, including low malaria endemic areas, including Gunung Mas, Pulang Pisau and Murung Raya Regencies (18). Based on the results of the study An. letifer was also confirmed as a malaria vector in Peninsular Malaysia with a sporozoite rate of 0.3-0.7%. Meanwhile, the results of a study in Sarawak Malaysia, *An. letifer* positive *Plasmodium* were non-human, but this species has the opportunity to be a vector of malaria in humans because it was found in large numbers due to land clearing activities (16). *Anopheles letifer* was also known to be a vector of malaria in humans and livestock in Malaya along with several species belonging to the group. *An. umbrosus* (19). Base on the study *An. letifer* as a vector in Malaysia with *An. maculatus, An. balabacensis, An. dirus, An. campestris, An. sundaicus, An. donaldi, An. leucophyrus* dan *An. flavirostris* (20). *Anopheles letifer* also as vector malaria in Singapura with *An. maculatus*, and *An. epiroticus*, (17),(21).

Anopheles letifer has the potential to be a malaria vector in the provinces of Riau, Bangka Belitung, South Kalimantan, Riau Islands, West Kalimantan, North Kalimantan, Jambi, and Central Kalimantan. This was because the survey results show that *An. letifer* Dominant were found to suck human blood rather than animal blood. The results of the study showed that 96.69% of *An. letifer* was found in the method of catching mosquitoes with human bait. Meanwhile, the results of the PCR examination also showed 100% *An. letifer* positively suck human blood. This condition shows that *An. letifers* have a close relationship with humans. The greater the chance of contact with humans, the more potential for malaria transmission. However, the percentage of this preference for sucking blood can vary from region to region. A study in Sarawak shows that *An. letifer* only 50% suck human blood (16).

An. letifer as a malaria vector can also be seen from its activity in sucking blood. *Anopheles letifer* has the opportunity to be a vector of malaria in several provinces in Indonesia because it has been found that blood-sucking activities occur throughout the night. This activity allows contact with humans to be greater which results in malaria transmission. Study in Serawak *An. letifer* was known to have blood-sucking activities from sunset until before 10 p.m. both indoor and outdoor (16).

The results of a study in Serian Selawak Malaysia found this species to be found throughout the night with a low population, with the peak density occurring at 24.00-01.00. *Anopheles letifer* was also found to be dominant in its population compared to other Anopheles species that were caught (22).

The mosquito density factor was also one of the factors that can increase the potential of *An. letifer* as a malarial vector. Based on the study of the population of the An. letifer highest were found in Jambi Province. Mosquito density was greatly influenced by climate which includes, temperature, humidity and rainfall (23),(24). Studies in Colombia and Venezuela show that malaria transmission was associated with increased temperatures, decreased rainfall and river conditions (25). Temperature also affects the process of parasite development. Meanwhile, rainfall can affect the formation of places of development of disease vectors (24). Based on a study of breeding sites, *An. letifer* were found in forest scrubs, and peat swamps (16).

Based on the analysis of behavior of *An. letifer* also pointed out that malaria transmission was most likely to occur outdoors. This was because An. letifers were found to be dominant in sucking human blood outside the house rather than indoors. A study in Serian Serawak, Malaysia also shows that An. Letifers tend to be found sucking blood outside the home rather than indoors (22). Seeing the behavior pattern of *Anopheles letifer*, the control of malaria vectors outdoor needs to be encouraged in order to reduce contact with humans outdoor. Studies in Sarawak also showed that *An. letifer* was predominantly found to suck blood outdoor when compared indoors with a ratio of 5:1 (16).

The dominant behavior of *An. letifer* found to suck blood outdoor, may be due to the success of the vector control program carried out using Indoor Residual Spraying (IRS) and insecticide mosquito nets. Based on a study in Africa, approximately 79% of mosquitoes are known to suck human blood while sleeping (26). With the intervention of vector control in the house, it will have an impact on the behavior of mosquitoes that usually suck human blood indoor to switch to sucking blood outdoor (22). This change in blood-sucking behavior can also be seen from *An. gambie* on Buoko Guinea Island, which was originally found to suck blood indoor, after the application of vector control with the IRS this species tends to suck blood outdoors (27). The application of vector control in rural areas of Tazania Africa using insecticidal mosquito nets and IRS causes a change in the population of vectors in the home that switch to outdoors, so that the transmission of malaria transmission in the home was reduced and replaced by transmission outdoor (28). The results of longitudinal survey studies in Kokofine and Mauno show that the application of vector control with insecticide mosquito nets causes mosquito activity in sucking blood to occur earlier before humans sleep in mosquito nets (29). Studies in Africa have also shown that changes in the behavior of mosquitoes sucking blood outdoors cause malaria transmission to occur more outdoors (26).

5. Conclusion

Anopheles letifer was distributed in Riau Province, Riau Islands, Jambi, Bangka Belitung, West Kalimantan, Central Kalimantan, South Kalimantan and North Kalimantan. *Anopheles letifer* has an all-night blood-sucking behavior and was dominant in sucking human blood, especially outdoors. Central Kalimantan was a province that has the potential for malaria transmission transmitted by *An. letifer*.

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