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ORIGINAL ARTICLE

Breeding Preference and Bionomics of *Anopheles spp.* at the Malarial Endemic Area, Runut Village, East Nusa Tenggara Province, Indonesia

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ABSTRACT

Introduction: *Anopheles* mosquito is transmitting malaria, one of the health problems in Indonesia. Understanding *Anopheles* mosquito behaviour and its breeding preference is one of the crucial keys to prevent malaria transmission. This study aimed to identify the breeding place distribution and bionomics of *Anopheles* spp. in Runut village, Sikka district, East Nusa Tenggara.
Methods: A descriptive observational study was conducted in Runut village, Waigete sub- district, in April 2018. *Anopheles* spp. larvae were collected in 7 suspected breeding places consisting of 2 rice fields, 3 fish ponds, and 2 puddles. Mosquitos behaviour was observed using bed-net traps located inside and outside the house from 7.15 PM to 1.15 AM after obtaining informed consent. Mosquito collection using bed-net trap were performed for 40 minutes then followed by resting mosquito collection for 10 minutes.
Results: *Anopheles* spp. larvae were found in most of the suspected aquatic habitats, presenting different densities and together with larvae of the other mosquito species. Relatively high number of *Anopheles* spp. larvae was obtained from a puddle. Only one female mosquito of *Anopheles* spp. resting on the wall inside house was found around 00.55 – 01.05 AM and resulted in low mosquito density determination.
Conclusion: *Anopheles* spp. larvae were harbouring in most of the aquatic habitats and one puddle contained moderately abundant larvae of *Anopheles* in Runut village, Sikka district, East Nusa Tenggara, Indonesia. Even only one *Anopheles* spp. mosquito was detected inside the house, residents in Runut village should regular use insecticide-treated bed nets and continuous observation of mosquito breeding places especially puddles to prevent malaria and other mosquito borne diseases.

Introduction

Malaria is a life-threatening vector-borne disease that is transmitted through the bites of infected female Anopheles mosquitoes. According to World Health Organization (WHO) in The World Malaria Report,[1] nearly half of the world's population lives in 87 malaria endemic countries with high risk of transmission and approximately 229 million malaria cases were reported in 2019, which is only slight declining from 238 million cases in 2000.[1] It also showed the malaria cases have declined globally by 27% between 2000 and 2015, however, there was a slowing rate of decline since 2015 for only 2% between 2015 and 2019. Moreover, the mortality rate in at-risk populations also decreased from 25 in 2000 to 12 in 2019 with the slowing rate of decline in the following years.[1] Indonesia holds the highest number of malaria cases in Southeast Asia countries and is becoming the second highest malaria cases worldwide after India.[1] Although the number of cases has decreased in 2010 to 2014, the trend tends to

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be stagnant in the next years. The Ministry of Health of Republic of Indonesia noted that the total number of malaria cases in Indonesia in 2019 was 250,644 and around 86% occurred in Papua.[2] Meanwhile, there are still around 214 regencies/cities (42%) that still have active malaria cases, which means around 59 million Indonesians live in malaria endemic areas.[2]

Malaria is one of the elimination target infectious diseases which enlisted in the Sustainable Development Goals (SDGs) as a global commitment that must be achieved by the end of 2030.[3] However, several regencies/cities in eastern Indonesia still belong malaria endemic area, including East Nusa Tenggara province. Although the national incidence rate of malaria has decreased from 1.4% in 2013 to 0.4% in 2018, it was reported East Nusa Tenggara yet ranked third with the most malaria cases in Indonesia.[4] Malaria morbidity rate, described by the Annual Parasite Incidence (API) indicator per 1,000 population, in East Nusa Tenggara accounts for 2.37 ‰ which is higher than the national API of 0.93 in 2019.[5] East Nusa Tenggara is one of the provinces that contributes the most malaria cases nationally.

Various malaria control programs to prevent new infections and to curtailed malaria transmission have been conducted globally for years. Vector control is one of the most important keys to control malaria and has been shown to be successful in reducing as well as preventing malaria transmission.[6-12] Long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) become the major pillar in the fight against malaria over decades.[13] However, despite universal use of LLINs and IRS deployment being implemented, in fact the malaria transmission still persists in many areas in Indonesia. This residual transmission might indicate a possibility that the standard insecticidal nets uses and residual spraying are considered inadequate and another strategy must be developed as Sherrard-smith E et.al. suggested in Africa cases.[14]

In this study, distribution of breeding sites and bionomic of *Anopheles* spp. in Runut village, Sikka district, East Nusa Tenggara were observed in April 2018. According to the Silla district health report, 11 malaria cases were detected in Runut village and 23 cases in Waigete sub-district (including Runut village) from January to April in 2018.

Methods

Study area and population

This study was conducted in Runut Village, where located in Waigete sub-district, Sikka District, East Nusa Tenggara province, Indonesia in April 2018 (Figure 1). Sikka District is an archipelago with 18 islands, nine of which are inhabited whereas others are uninhabited. Sikka District has an area of 7,552.91 km^2 consisting of 1,731.91 Km^2 of land area and 5,821 km^2 of sea area. The topography is mostly hilly, mountainous, and valley with steep slopes which are generally located in coastal areas. This district has tropical climate similar with other areas in Indonesia in general. The average temperature is 27.2°C with average humidity 85.5% and average wind speed 12–20 knots per year. Sikka Regency is bordered by the Flores Sea to the north, Sewu Sea to the south, East Flores District to the east, and Ende District to the west. Administratively, this district consists of 21 sub-districts covering 147 villages.[15]

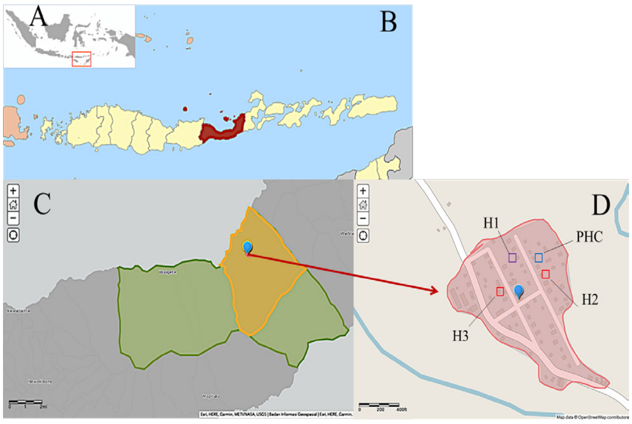

Figure 1. Runut village (orange in C) is located in Waigete sub-district (green in C), Sikka district (red in B). Blue point indicates Lodong sub-village (rose in D, arrowed), where study was conducted (source: arcgis.com16). Location of the houses presented in Figure 2B are shown.

Runut is one of the largest villages in the Waigete sub-district which located in area of 233.84 km^2. In 2018, the population in Waigete sub-district is 23,077 people consisting of 11,002 men and 12,075 women with a population density of 181 people per km^2. Runut Village locates in 780 meters above sea level and is the highest village in the sub-district. Waigete sub-district is bordered to the north by the Flores Sea, Bola sub-district to the south, Talibura sub-district to the east and Waigete sub-district to the west.[15]

Study design and data collection

A cross-sectional observational descriptive study was applied in Lodong sub-village, Runut village, where the residential is not densely settled and the distance between one house and others is quite far. The area is surrounded by forests and has one auxiliary primary health care center. The mosquito catching was carried out in a resident's house after receiving their written informed consent approval. The collection of larvae was carried out at seven suspected location points, which are two puddles, two rice fields and three fish ponds. One of the rice fields is located at an altitude of about 300 meters above sea level. One of the puddles with size of roughly 50 x 60 cm was beside a small path. In Figure 2B, the houses where the residents had been suffered from malaria are presented with red color.

Entomological Surveillance and Species Identification

Breeding site observation

The collection of larvae was carried out by squeezing technique and the obtained larvae were put into a container for calculation of the density. Larvae sampling was performed ten times for each breeding site according to the standard operating procedure17-19. The larvae density was calculated based on WHO 1975 (the number of larvae caught in each type of breeding site per scoop)19. The density number is said to be high if in one scoop found 20 or more larvae.

Mosquitos' collection technique

Mosquitos' collection was conducted at night for 6 hours from 18.00 till 24.00. It is consisted with every one hour of 40 minutes for catching mosquitoes both indoor and outdoor using human-baited tent (a double-net) traps, 10 minutes for collection of resting mosquitos both endophilic and exophilic, and 10 minutes for collector's resting time and let mosquito trap inside tent. The bed-net trap technique was performed in accordance to WHO standards (1975).[19]

The human-baited tents with a villager inside each tent were designated by the double-net to catch mosquitos. The traps used tight and strong nets and the nets used must be larger than the bed or animal-baited. The net was placed hanged and a hole with a size of 15-20 cm was made between the floor and the net for mosquitoes to enter the trap. An aspirator was used to collect mosquitoes which then obtained and put into a modified cup with gauze and cotton covered the top of the cup. Man-hour density (MHD) and man biting rate (MBR) were used to estimate mosquitos' density.

Ethical approval

This study was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Airlangga (310/EC/KEPK/FKUA/2018).

Results

Anopheles spp. Larvae Breeding Places

Seven locations, consisting of two puddles, three fish-ponds and two rice fields were observed as the possible breeding sites for *Anopheles* spp. larvae. (see on Figure 2A and Figure 2B). At each aquatic habitat, ten intakes (scoops) were carried out and *Anopheles* spp. larvae were found in most of the locations. Total of 135 *Anopheles* spp. larvae were obtained from six locations. Most of them (99 larvae) were from the puddle 2 and several larvae were from the other settings except puddle 1 (Table 1). It is noteworthy that the puddle 2 is close to the house (H3) of prior malaria infected patient (Figure 2B). The other mosquito species larvae were detected in most of the suspected locations with varying densities (Table 1).

Anopheles spp. bionomics

Bionomic distribution of *Anopheles* spp. were assessed based on the time and place of collecting. The mosquito collection was conducted at the house 1 (H1, Figure 3) of both indoor and outdoor using human-baited tent traps at night. No *Anopheles* spp. mosquito was found from neither indoor nor outdoor collection sites. The other types of mosquitos were detected during this collection periods. *Culex* spp. were obtained throughout the collection time in the indoor and outdoor bed-net traps whereas *Aedes* spp. and *Mansonia* spp. were dominantly found from the outdoor bed-net trapping at relatively early night time (Table 2).

Resting mosquitoes on the walls of bed room were collected for 10 minutes every one hour, and obtained one *Anopheles* spp. at 00.55-01.05 and one *Culex* spp. at 19.55–20.05 (Table 2B). Based on this mosquito detection frequencies, the mosquito density of *Anopheles* spp. represented by man hour density (MHD) was 0.04 mosquitoes/person/hour and 0.25 mosquitoes/person/night by man biting rate (MBR). *Culex* spp. was detected throughout night time at both indoor and outdoor collection sites (Table 2A).

Table 1. Distribution of mosquito larvae in breeding places

Code	Breeding place	Mosquitos' genus			
		Aedes spp.	*Anopheles spp.*	*Culex spp.*	*Mansonia spp.*
P1	Puddle 1	0	0	18	0
F1	Fish pond 1	0	1	33	0
F2	Fish pond 2	0	11	32	0
R1	Rice field 1	0	6	0	0
R2	Rice field 2	0	9	2	0
P2	Puddle 2	0	99	1	0
F3	Fish pond 3	0	9	4	0
	Total	0	135	90	0

Table 2. Distribution of mosquitoes indoors and outdoors.

Observation time	Indoors				Outdoors			
	Aedes spp.	*Anopheles spp.*	*Culex spp.*	*Mansonia spp.*	*Aedes spp.*	*Anopheles spp.*	*Culex spp.*	*Mansonia spp.*
19.15 -19.55	0	0	1	0	2	0	1	0
20.15 -20.55	0	0	1	0	0	0	0	2
21.15 -21.55	0	0	2	0	0	0	1	3
22.15 -22.55	0	0	2	0	0	0	1	0
23.15 -23.55	0	0	0	0	0	0	0	0
00.15 -00.55	0	0	1	0	0	0	1	0
Total	0	0	7	0	2	0	4	5

Table 3. Distribution of mosquitoes during resting time

Observation time	Resting mosquitoes			
	Aedes spp.	*Anopheles spp.*	*Culex spp.*	*Mansonia spp.*
19.55 - 20.05	0	0	1	0
20.55 - 21.05	0	0	0	0
21.55 - 22.05	0	0	0	0
22.55 - 23.05	0	0	0	0
23.55 - 00.05	0	0	0	0
00.55 - 01.05	0	1	0	0
Total	0	1	1	0

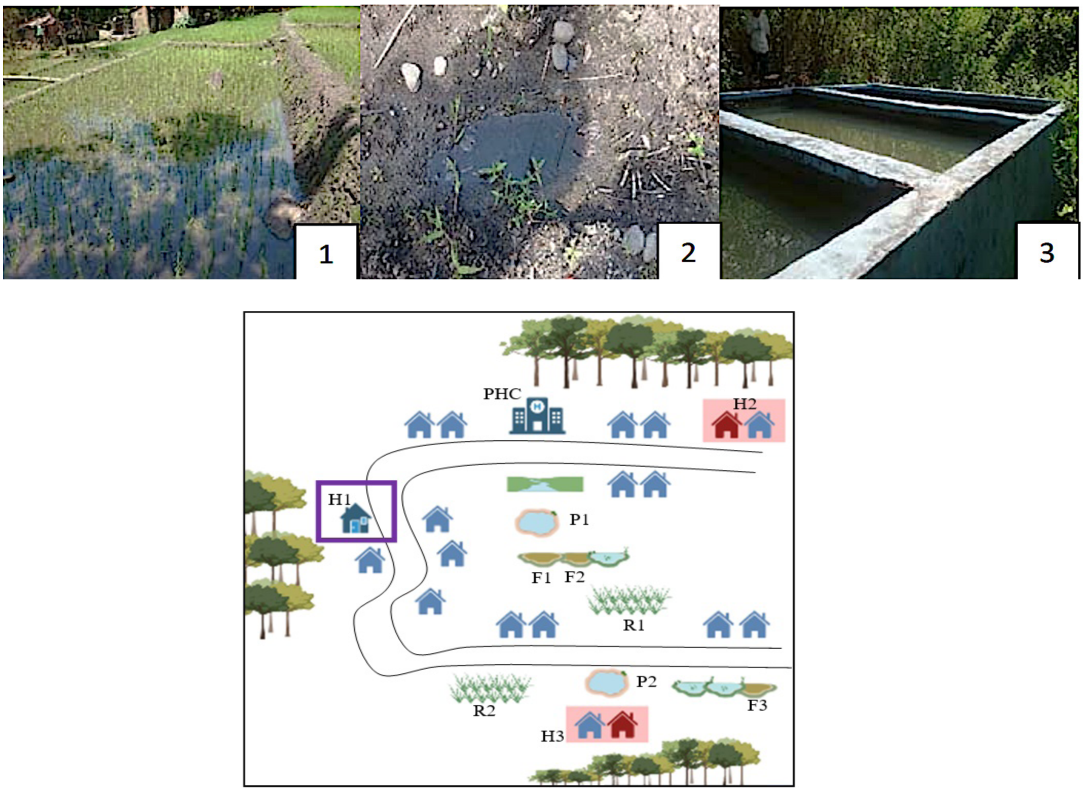

Figure 2. (A) Mosquito larvae collecting sites are; 1) rice field, 2) puddle, and 3) fish-ponds ; (B) Location of the suspected Anopheles spp breeding places. H1 is the house conducted for mosquito collection in this research, and H2 and H3 are the houses of which residents were experienced malaria infection. PHC is the auxillary primary health center.

Discussion

In this Anopheles spp. mosquito and larva collection research in Runut village of Sikka district, several *Anopheles* spp. larvae were detected in the most of the suspected aquatic habitats and relatively high number of *Anopheles* spp. larvae were collected from one of the puddle (Puddle 2), close to the house (H3) of prior malaria infected patient. The larvae of the other mosquito species were also observed in these aquatic habitats. These mosquito larvae detection suggests many of the aquatic locations are mosquito breeding places, including *Anopheles* spp. and regular observation and reduction of larvae is important for the control of malaria and mosquito transmitted infectious diseases.

Mosquito larvae density is significantly influenced by physical and biological factors of the habitat and environment. Different densities of *Anopheles* spp. larvae ware noticed among the observed seven aquatic habitats, especially between Puddle 1 and Puddle 2, no Anopheles spp. larvae detected in Puddle 1 and abundance of *Anopheles* spp. larvae in Puddle 2. The Puddle 1 is an artificial puddle used for bathing pigs and containing some tin-head fish (Aplocheilus panchax), while the Puddle 2 is near a small path and no fish in there. Aplocheilus panchax is well known as the natural predator of mosquito larvae and the prey consumption is varied significantly depending on habitat and prey type.[20] In the case of Puddle 1, different effects on *Anopheles* spp. larvae and *Culex* spp. larvae were observed: no *Anopheles* spp. larvae were detected were observed even some numbers of *Culex* spp. larvae. Further study of the larvivorous fish Aplocheilius panchax on the effect of *Anopheles* spp. is worth investigating for malaria control. What kind of factors, habitat type, water temperature, surface vegetation, and the others, are important to reduce *Anopheles* spp. larvae.

Only one Anopheles spp. mosquito were obtained at resting time on the wall of bedroom (Table 2A and 2B).

Bionomics of *Anopheles* spp. in Sikka district has not been comprehensively explored yet. But our observation and some previous local information suggested the endophilic behaviour of *Anopheles* spp, a risk of indoor malaria transmission in Runut village. Considering detection of *Anopheles* spp, larvae in most of the aquatic habitats, use of insecticide bed nets is essential for avoiding the indoor malaria transmission.[21-25]

Regular observations of vector behaviour and larval breeding sites are important in malaria endemic areas to notice a change of vector populations and breeding sites at any time and every place.[26-29] Environmental changes such as deforestation could increase local temperatures in the highlands that could enhance the vectorial capacity of the *Anopheles*.[30-32] In Sikka district East Nusa Tenggara, a possibility of occasional changes of potential vectors, seasonal vector changes were reported by Majawati et.al that Anopheles barbirostris was a dominant malaria vector during dry season whereas An. aconitus predominated during rainy seasons.[33] The other studies in the same Sikka district suggested minimal changes in dominant Anopheles species. Systematic vector analysis is required for efficient malaria control strategies in the still malaria endemic in Sikka district, East Nusa Tenggara Province.

Conclusion

In this research in Runut village, Sikka district, East Nusa Tenggara, Indonesia, several Anopheles spp. larvae were collected in most of the aquatic habitats and one of the puddles was moderately abundant. Even only one Anopheles spp. mosquito was detected inside the house, residents in Runut village are recommended to use insecticide-treated bed nets and continuous observation of mosquito breeding places especially puddles to prevent malaria and other mosquito transmitted diseases.

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Conflict of Interest

Declare no conflict of interests for this article.

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