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**Different Types of *Anopheles* Breeding Place in Low and High Malaria Case Areas**

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Abstract

Malaria is a disease which is transmitted through the bite of *Anopheles* mosquito. This study aimed to analyse the difference of type of *Anopheles* larva breeding places between low malaria case area, namely Mandor Village, and high malaria case area, namely North Amboyo Village, both in Landak Regency, West Kalimantan Province. The samples of *Anopheles* mosquito breeding places was acquired through accidental sampling from all *Anopheles* larva breeding places and spatial mapping of breeding place points. The data were analyzed using chi-square test and Mann-whitney test. This study found 70 breeding place points which consisted of 8 types of place, namely dig well, drilled well, Illegal Gold Mining (PETI) well, puddle, fish pond, sewer, swamp, and rice field. The lowest larva density in Mandor Village was at PETI well (0.48/dip) and the highest was at dig well (0.75/dip). In North Amboyo Village, the lowest density was at number 3 dig well (0.2/dip) and the highest was at rice field (2.3/dip). It can be concluded that there was a significant difference of *Anopheles* breeding places between low and high malaria case areas.

Introduction

West Kalimantan is a province with numerous malaria endemic regencies, namely Kapuas Hulu Regency, Bengkayang Regency, Sintang Regency, Ketapang Regency, Sanggau Regency, and Landak Regency. In 2009, there was an outbreak of malaria in West Kalimantan, namely in Wajok Subdistrict of Mempawah Regency. The number of malaria cases in Landak Regency in 2015 was 3566 cases of clinical malaria and 41 cases of microscopic positive malaria. In 2016, there was 4409 cases

of clinical malaria and 15 cases of microscopic positive malaria. In 2016, the annual parasite incidence (API) number in Landak Regency was 0.04 per 1000 people while in West Kalimantan Province, the number was 0.07 per 1000 people. Clinical data from laboratory examination of malaria cases found *P. falciparum* and *P. vivax* parasites (Dinas Kesehatan Kabupaten Landak 2017).

Mandor Village was an area with low malaria cases with the number of clinical malaria cases were 47 cases in 2016 and 24 cases

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in 2017 (Puskesmas Mandor, 2018). North Amboyo Village was an area with high malaria cases with the number of clinical malaria cases was 58 cases in 2016 and 30 cases in 2017 and the number of microscopic positive cases were 15 cases in 2016 and 6 cases in 2017 (Puskesmas Semata, 2018). According to information from Health Agency of Landak Regency, malaria cases which occurred in North Amboyo Village were import cases from people from Papua which resided in North Amboyo Village, Ngabang Subdistrict, Landak Regency, West Kalimantan Province.

The majority of people in Landak Regency is Dayak tribe. The area consists of mountainous and high hill area with variation in altitude. High intensity rainfall is influenced by the presence of tropical forest. Meteorology, Climatology, and Geophysical Agency (BMKG) in Mempawah recorded that climatological condition of Landak Regency showed average rainfall between 245-350 mm/month in 2018 (BMKG Mempawah, 2018). The majority of residents in Mandor Village worked as farmer, rubber plantation worker, Illegal Gold Mining (PETI) worker, and private worker. Meanwhile, the majority of North Amboyo Village residents worked as farmer, oil palm plantation worker, horticultural and rice farmer (BPS Kabupaten Landak, 2017).

The environment of Landak Regency is very suitable as habitat for malaria vector. Hakim (2011) and Amani et al., (2014) stated that coast, rice field, forest, and mountain areas were suitable environment for *Anopheles* reproduction. The type of *Anopheles* breeding places could contain different *Anopheles* species. Characteristics of breeding places could be correlated with the environment. Several studies concluded that there were correlation between characteristics of *Anopheles* larva habitat and environment towards larva density which could be used as basis to determine malaria elimination program (Jastam 2014; Soleimani-Ahmadi et al., 2013; Tulak et al., 2018).

Spatial analysis is a technique/process which involves several calculation and evaluation of mathematical logics in order to find the relationship or pattern which exist between spatial components. This

spatial analysis was used to see geographical description using basic map of Mandor Village, Mandor Subdistrict and North Amboyo Village, Ngabang Subdistrict by determining the coordinate points using Global Positioning System (GPS) equipment. The signal which is emitted by those satellites are information regarding the satellite's position which is continuously and simultaneously emitted to signal receiver on earth which then be processed to become coordinate information which could be globally known by every person with clear measurement and coordinate system. This study was conducted to analyze the difference in characteristic of breeding place which was correlated with larva density and to understand geographical description of breeding place in both study areas.

Methods

This was an observational analytic study with cross sectional design. This was an observational study because it aimed to describe the phenomena which were found, particularly the bionomic of malaria vector which was conducted using spot survey method on the field. This study was conducted in March 2018. The location were area with low malaria case, namely Mandor Village, Mandor Subdistrict and area with high malaria case, namely North Amboyo Village, Ngabang Subdistrict, Landak Regency, West Kalimantan Province. The discovered breeding places were then observed in order to know the presence of *Anopheles* larvae. The capture of larva in breeding place used dipper with capacity of 320 mL and maximal samples of 10 standard dip. The used dipper was appropriate with WHO standard with volume of 250-350 mL. Larva capture was conducted at 7 am to 5 pm WIB (Indonesian Western Time). The captured larvae were counted in order to know the larva density in each breeding place.

The data were analyzed using Statistical Package for the Social Sciences (SPSS) with Chi-square test and Mann-Whitney test. Spatial analysis was performed in order to know the mapping distribution of breeding places in both study areas using GPS equipment with Geographical Information System. Area/buffer with certain distance from spatial element would be detected by signal network of

coordinate point automatically in Garmin 78s equipment.

Results and Discussion

The difference of breeding places description in Mandor Village was determined as 6 types, namely PETI well, swamp, dig well, puddle, sewer, and fish pond. In North Amboyo, there were also 6 type of breeding places with slight different type from the first village, namely swamp, dig well, rice field, sewer, drilled well, and puddle. Overall, there were 8 type of *Anopheles* larvae breeding place in Mandor Village and North Amboyo Village, namely PETI well, dig well, swamp, drilled well, sewer, puddle, fish pond, and rice field. For Chi-square analysis, the breeding places were differentiated into well category and non-well category. Well category consisted of 4 breeding places, namely PETI well, dig well, drilled well, and fish pond, while the rest of breeding places were included in non-well category, namely puddle, swamp, rice field, and sewer. The distribution of *Anopheles* larva breeding places in Mandor Village and North Amboyo Village are shown at Table 1.

Table 1 showed that the most common *Anopheles* larva breeding place in Mandor Village (low malaria case) was well (27 place/67.5%), while the most common breeding place in North Amboyo Village (high malaria case) was non-well (19 place/63.3%). Chi-square analysis found p-value of 0.010 which meant that there was a significant difference of *Anopheles* larva breeding place in both village (p<0.05). In area with low malaria cases, the most common breeding place were PETI well (well category) and swamp (non-well category).

This finding was different from area with high malaria case where the most common breeding place was swamp (non-well category). In both location, the majority of breeding place was different therefore the characteristic of breeding place in both village were also different.

Mandor Village is located in lowland which is often used in illegal gold mining (PETI). The habit of leaving holes after the illegal gold mining has ended produced puddles which are similar to well and it could become *Anopheles* larva breeding place. This type of breeding place could also be found in Buluh Kuning Village, Kotabaru Regency, South Kalimantan Province (Indriyati et al., 2017).

North Amboyo Village is mostly located in forest and oil palm plantation. The type of breeding places in this village was more variable but the most common was swamp (10 places/33.33%). In North Amboyo Village, there were numerous puddles in oil palm plantation, oil palm waste, and swamp canal in the forest which were breeding place and were located not far from people house. A similar finding was found by Sandy *et al.*, (2017) who stated that *Anopheles* breeding place could be found in oil palm plantation. Breeding places which were located in oil palm plantation were also became protector from sun light. Bushes and protector plant also functioned as resting place for mosquito which had developed from aquatic phase. Those plant commonly functioned as protector to cover the habitat, hence they did not directly exposed to sun light which could increase water temperature (Sánchez-Ribas et al., 2017).

Human activity in construction such

Table 1. Distribution of *Anopheles* Larva Breeding Places in Mandor Village and North Amboyo Village, Landak Regency, West Kalimantan Province

Type of breeding place	Malaria Case Group				Total	%	p*
	Low malaria case (Mandor Village)		High malaria case (North Amboyo Village)				
	N	%	N	%			
Well	27	67.5%	11	36.7%	38	54.3	0.010
Non-well	13	32.5%	19	63.3%	32	45.7	
Total	40	100	30	30	70	100	

*p<0.05 Fisher's exact test

as neglected fish pond, dam construction, tin mining, and land opening for agricultural and veterinary purpose caused environmental changes which could produce man made breeding places. Man-made breeding places with limited water could become an important factor in increase of *Anopheles* presence in endemic area in Amazon (Rufalco-Moutinho et al., 2016). The results of human activity produced numerous place which were suitable for development of malaria vectors, for example well, sewer, puddle which was filled with rain water, and rice field with irrigation flow (Djati et al., 2011; Indriyati et al., 2017; Jastam, 2014; Sugiarto et al., 2016). Other than that, temporary puddle and small river with slow flow could also be found as malaria vector breeding places (Ariati et al., 2014).

Most of the residents in North Amboyo Village worked as oil palm plantation worker and lived inside the plantation. Forrest opening for the purpose of producing oil palm plantation had several effects on worker and environmental health, namely disturbing the natural habitat of *Anopheles* mosquito. Worker at oil palm plantation were at risk to be bitten by *Anopheles* mosquito because the worker walked through small path above grassy bushes which ran through *Anopheles* mosquito breeding

place, therefore they were at higher risk to be infected by malaria (Sukowati, 2008). This was the cause of high malaria cases in this village.

Area with different environmental condition showed different breeding place. Population of *Anopheles* sp. would be fluctuated according to the seasonal vegetation dynamics (Rueda et al., 2010). In this study, both Mandor Village and North Amboyo Village had different types of breeding places. Another example was in Sebatik Island, Nunukan Regency, North Kalimantan Province which had lagoon, fishpond, sewer, and swamp as breeding places (Sugiarto et al., 2016). In Rudan County, which is located in southeastern part of Iran, sand mining pond and clean water puddle could also be found as breeding places (Soleimani-Ahmadi et al., 2013).

The breeding places which were found to positively contain *Anopheles* larva in Mandor Village were PETI well and dig well. Meanwhile, in North Amboyo Village, the breeding place which positively contained *Anopheles* larva were sewer, number 1 dig well, number 1 swamp, number 2 dig well, rice field, drilled well, number 2 swamp, number 3 dig well, and number 2 swamp. This findings were in accordance with previous study which found that case group most commonly lived near the

Table 2. *Anopheles* Larva Density in Landak Regency, West Kalimantan Province

No.	Type of breeding places	<i>Anopheles</i> Larva Density		
		Number of Dip	Number of Larva	Density
Mandor Village				
1.	PETI well	25	12	0.48
2.	Dig well	20	15	0.75
North Amboyo Village				
1.	Sewer	10	3	0.3
2.	Number 1 dig well	10	11	1.1
3.	Number 1 swamp	10	6	0.6
4.	Number 2 dig well	10	6	0.6
5.	Rice field	29	65	2.3
6.	Drilled well	18	8	0.4
7.	Number 2 swamp	10	6	0.6
8.	Number 3 dig well	20	4	0.2
9.	Number 3 swamp	16	14	0.9

swamp and garden, had the habit to go out at night, and did not use mosquito net and anti-mosquito (Mangguang, 2015). Larva density in each breeding places are shown at Table 2.

Table 2 showed that the number of larva which were found in Mandor Village during the study were 23 with the lowest density was in PETI well (0.48/dip) and the highest density was in dig well (0.75/dip). This was possibly because PETI well had been contaminated by chemical substance such as mercury and it had turbid water, while in dig well, there was no chemical contamination and the water was relatively clear. Therefore, PETI well could only be inhabited by certain *Anopheles* species (Indriyati et al., 2016).

The number of larva which were found in North Amboyo Village during this study were 123 with various larva density ranged from 0.2/dip until 2.3/dip. The highest density was found in rice field (2.3/dip) and the lowest density was found in number 3 dig well (0.2/dip). Rice field and irrigation canal were liked by malaria vector as places for reproduction because there were water plants which could be used as protection for larva (Mading, 2013; Mading & Sopi, 2014; Yulidar, 2017).

This study also found that swamp had relatively high larva density. Willa and Kazwaini (2015) predicted that this might be caused by the presence of grass and moss in swamp which made them liked by malaria vector as place for reproduction. Other than that, other factors which supported the reproduction of malaria vector were pH, salinity, water turbidity, and sun light exposure (Mading & Sopi, 2014; Sari, 2018).

Normality test using One-sample Kolmogorov-Smirnov test on larva density in Mandor Village and North Amboyo Village showed that the data were normally distributed (Table 3). When all of the breeding places,

whether they contained larva or not, were analyzed using Mann-Whitney test, it could found that there was a significant difference of *Anopheles* larva density between area with low malaria cases and area with high malaria cases. The result of Mann-Whitney test found p-value=0.001 (p<0.05) which meant that there was a significant difference in *Anopheles* larva density between Mandor Village and North Amboyo Village. This difference might be caused because of difference in geographical characteristics between both study areas which had been explained before. Mandor Village is a mining area while North Amboyo Village is a plantation area.

Although there was a difference in *Anopheles* larva density between the two villages, both villages show density value >1. This condition were higher than normal value which has been determined by Ministry of Health Regulation No. 50 Year 2017 regarding Standard of Quality of Environmental Health and Health Requirements of Vector and Animal which Carry Diseases. Therefore, the government should pay attention to malaria control in this region.

The results of mapping spatial analysis of breeding places distribution in area with low malaria cases (Mandor Village) showed 5 coordinate points where there were breeding places with positive presence of *Anopheles* larva and 35 coordinate points where there were breeding places with negative presence of *Anopheles* larva. Meanwhile, in area with high malaria cases (North Amboyo Village), there were 15 coordinate points of breeding places with positive presence of *Anopheles* larva and 15 coordinate points of breeding places with negative presence of *Anopheles* larva. The map of breeding places in both villages are shown in Figure 1 and Figure 2.

Spatial analysis through clustering

Table 3. The Difference in *Anopheles* Larva Density between Area with Low Malaria Case and Area with High Malaria Case in Landak Regency, West Kalimantan Province

	Malaria Area Category	N	Average	p*
<i>Anopheles</i> Larva Density	Low malaria case	40	29.79	0.001
	High malaria case	30	43.12	
	Total	70		

*p<0.05 Fisher's exact test

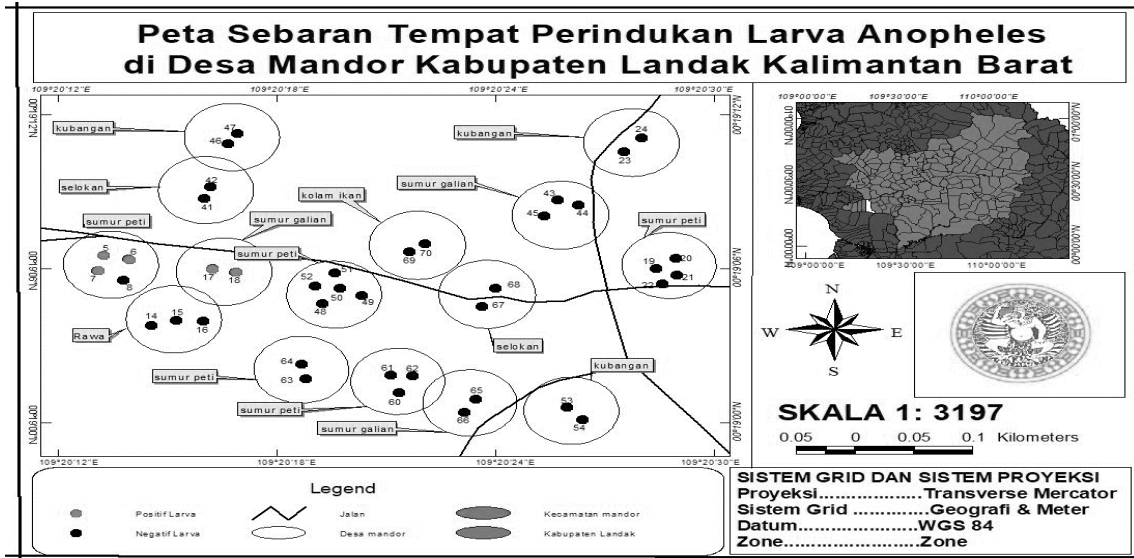


Figure 1. Map of Distribution of Breeding Places in Area with Low Malaria Cases in Mandor Village, Mandor Subdistrict, Landak Regency, 2018



Figure 2. Map of Distribution of Breeding Places in Area with High Malaria Cases in North Amboyo Village, Ngabang Subdistrict, Landak Regency, 2018

mapping of breeding places distribution according to breeding places showed $p=0.001$ which meant that the clustering was significant. When there were cases of malaria near the cluster, there would be an increase in risk of malaria transmission in that cluster area.

Numerous measures had been conducted to eradicate malaria. Those measures need people's participation. Systematic review regarding risk factors of malaria diseases in Indonesia which was conducted by Setiyabudi

(2016) supported the finding of a study by Kalsum et al., (2015) which found that breeding places was one of 21 risk factors which found to have correlation with prevalence of malaria.

Healthcare personnel should ask the people to actively participate in reducing breeding places, for example by cleaning the bushes, piling holes which had the potential to become puddle during rain and by routinely cleaning the sewer/gutter, therefore the water flow would become smoother. People should

also implement biological control measures through keeping several animal such as tin head fish (*A. panchax*), Rohu (*Labeo rohita*), Catla (*Catla catla*), and Mrigal (*Cirrhinus mrigala*). Those fishes could be useful as mosquito control measure in certain situation (Kant et al., 2013).

Conclusions

There were 70 *Anopheles* larva breeding places which were discovered in both village. The breeding places were classified into 8 types, namely dig well, drilled well, Illegal Gold Mining (PETI) well, puddle, fish pond, sewer, swamp, and rice field. The density of *Anopheles* larva which were captured in each location were different because the number of larva was influenced by ideal environment and weather condition which affect *Anopheles* mosquito reproduction, therefore the distribution of breeding places in area with different number of malaria cases and different environment would showed different type of breeding place.

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