



ENTOMOLOGICAL SURVEILLANCE OF MALARIA VECTORS IN SAUMLAKI, MALUKU TENGGARA BARAT REGENCY, MALUKU PROVINCE

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Abstract

The research aims to determine the prevalence of malaria and *Anopheles spp* using biocology surveillance in Alusi and Waturu community health centers in Maluku Tenggara Barat Regency. The study was conducted in March-April 2015 with cross sectional design. In this research, we performed mass blood survey on 489 participants in the Kilmasa village and 434 participants in Waturu village. We also performed entomology surveillances, i.e. larval density, catching *Anopheles* spp, temperature, humidity, and salinity. To confirm malaria vectors, we used enzyme linked immunoabsorbent assay (ELISA) techniques. The data analyzed descriptively. The results of the study showed proportion 0.20% malaria morbidity in Kilmasa village and 0.23% in Waturu village. *Anopheles flavirostris* and *An. barbirostris* group were likely to bite a human outside and inside the house and peaked at 11.00 pm-12.00 pm. The parous rate of *An. flavirostris* and *An. barbirostris* was 46% and 26%, respectively. Human blood index of *An. flavirostris* and *An. barbirostris* was 33.3% and by 70%, respectively. *Anopheles flavirostri* and *An. barbirostris* were malaria vectors with sporozoite rate 0.38% and 12.5%, respectively.

Introduction

Malaria is a disease that is considered dangerous in the world. Each year 300 to 500 million cases of malaria is reported with mortality rate 2.7 million people worldwide, mostly occur in children. Indonesia is among countries with the highest malaria prevalence after India in South Asia. This disease may lower the health status and population productivity so it consequently to be a major burden to social and economic development (Rahmawati, 2014). Malaria is transmitted by mosquito vector (*Anopheles*), which are mostly found in a swamp area. In Indonesia, the disease is an endemic disease that is prevalent in many communities, due to this disease has long time

suffered many individuals in coastal areas, rice fields, plantations, and forest areas (Mardiana 2009). Malaria endemic area is high especially in the eastern part of Indonesia which is generally a remote area with lower socioeconomic status, unfavourable environmental conditions and lack of transportation facilities. Many malaria cases were reported from eastern Indonesia (Papua, West Papua, East Nusa Tenggara, Maluku and North Maluku) (Dijend PP dan PL, 2014).

Maluku province is a malaria endemic area with Annual Parasitic Incidence (API) morbidity 12.3/1000 population in 2008, decrease by 7.0/1000 population in 2009, and then increase again to 10.4/1000 population in

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2010, 9.1/1000 population in 2011, 11.1/1000 population in 2012 and 11.5/1000 population in 2013. High malaria endemic areas (API > 1 ‰) in Maluku province are include Southwest Maluku, Southeast Maluku, West Seram, Central Maluku, East Seram. Moderate endemic areas (API 1-10 ‰) are include Buru Island, Central Maluku, Ambon and Tual (Maluku, 2014). Maluku Tenggara Barat Regency selected as the research location because this area has high number of malaria cases with API 15‰ in 2013. This town is the third highest cases after West Seram Regency (API 22 ‰) and Southwest Maluku Regency (API 20 ‰) (Dinkes Maluku, 2014). The prevalence of malaria vector mosquito *Anopheles* in Maluku Tenggara Barat Regency has not been much studied yet. The mosquito *Anopheles spp.* distribution influenced by various environmental factors, including the physical environment such as weather conditions, geography, land use; and the microenvironment such as puddles for laying eggs and breeding habitat (Suwito, 2010). To determine the strategy to prevent transmission of malaria in Maluku Tenggara Barat Regency, it is required comprehensive and intensive efforts by public health office through maximizing the use of basic epidemiological data (agent, host and environment), bionomic aspect malaria vector and environmental factors in endemic malaria, accurately and specifically, therefore effective and efficient interventions can be delivered (Santoso, 2010).

This research was conducted to obtain information of the malaria prevalence and bioecology of mosquito *Anopheles spp* in Saumlaki, Maluku Tenggara Barat Regency.

Method

Entomology surveillance study was conducted at community health center with highest morbidity of malaria which were Alusi Kelaan and Waturu community health center in Saumlaki, Maluku Tenggara Barat, Maluku Province during March-April 2015. The research has had approval from the Commission on Ethics Board of Health Research and Development (No. Letter: LB.02.01 /5.2/KE.157/2015), and got research permission from the National Unity and Political of Maluku (Letter Number: 074/50/BKBP/II/2015). The study used observational

descriptive study with cross-sectional design and used spot surveillance method. Mosquito larvae surveillance was carried out to all the potential puddles as laying eggs spot for adult mosquito, the larvae was captured by scooping the water with 10 times detention. Captured larvae then inserted into the labelled tube and taken to the field station to be preserved or identified

$$\text{Larval density/larvae} = \frac{\text{NUMBER OF CAPTURED LARVAE}}{\text{NUMBER OF DETENTION}}$$

Prior to surveillance data collection for adults mosquito *Anopheles spp*, we conducted a preliminary study to randomly capture mosquitoes in community houses. Houses that qualify for mosquito bionomics observation selected as sample study (4 houses). The mosquito was captured inside and outside the house (indoor and outdoor landing collection) to determine the *man biting rate* (MBR). Mosquitoes captured by man landing indoor and outdoor collection was held in the evening at 6:00 pm to 06:00 am, performed by four people, two people inside the house and other two people outside the house for 45 minutes and rest for 15 minutes (Suwito, 2010). Mosquitoes captured in the morning performed at 6:00 a.m. to 8:00 am. Mosquitoes captured in the morning included catching mosquitoes that rest inside houses or other buildings in the morning and was done by two people, each captured mosquitoes in 4 houses for 15 minutes. Mosquito that rested outside the house was captured by two people. The mosquito was captured on a grass/vegetation, river banks, irrigation canal, ditches etc. Mosquitoes that rested captured in or around the livestock barns, carried out by one person mosquito catcher. The capture carried out in the existing livestock barns for 15 minutes per cage (Barodji, 2000; Mading, 2014). The captured-mosquitoes were then identified. The calculation for MBR and MHD are as follows:

$$\text{MBR} = \frac{\text{NUMBER OF MOSQUITO SPECIES CAPTURED}}{\text{NUMBER OF CATCHER X DURATION (DAYS)}}$$

$$\text{MHD} = \frac{\text{NUMBER OF MOSQUITO SPECIES CAPTURED}}{\text{NUMBER OF CATCHER X DURATION}}$$

Some of *Anopheles spp.* that obtained from the study area was examined by ELISA techniques to determine *Plasmodium spp* species by looking at the sporozoites. To

detect the presence of sporozoites, we used a monoclonal antibody *P. falciparum* (Pf 210) and *P. vivax* (Pv 210). Mass Blood Survey (MBS) surveillance was performed at peripheral blood with microscope in the study sites to determine the presence of malaria parasites in the blood (Kazwaini 2015). The test used equipment and materials such as glass object, sterile lancet, cotton, 70% alcohol, buffer tablet, 5% Giemsa, oil immersion and the compound microscope (Nikon).

Results and Discussion

Alusi Kelaan is the capital of the Kormomolin subdistrict where the Alusi community health center is located. The total population in the Kormomolin subdistrict is about 6.108 people with the most population work as farmer and fisherman. An illustration of malaria disease situation in Alusi and Waturu community health center can be seen in Figures 3 and 5. Kilmasa and Kelaan are two malaria endemic villages, where the Annual Parasite Index (API) of Kilmasa village has increased in 2011-2013, from 10.14 to 103.43 and 16.27 to 82.93, respectively. Waturu community health center is located in Nirunmas subdistrict with capital Tutukembong. This subdistrict has five administrative regions: Waturu village, Tutukembong village, Maglusi village, Arma village and Watmuri village. Waturu and Tutukembong village are the areas with a fairly high incidence of malaria.

An illustration of the Annual Parasite Index (API) can be seen in Figure 4, where a decrease occur in 2013 to 2014 in two villages i.e. Waturu village (19.33 to 10.54) and Tutukembong village (16.58 to 8.90). The results of Mass Blood Survey (MBS) in April 2015 with the thick blood smear method in Kilmasa village obtained 489 samples slide and after examination *P. vivax*, we found positive in one sample slide. Similarly, in the Waturu

village the MBS were collected 434 samples of thick blood smear and *P. vivax* were found positive in one sample.

The MBS results indicated malaria cases decrease in April due to a month ago before the study conducted, Maluku Tenggara Barat Public Health Office had done malaria treatment program in February and March due to increased cases of malaria those months. Hence, when the Public Health Office conducted an evaluation by MBS, the malaria cases already low, and at the time of MBS by the research team were also the cases have dropped to only 1 case of malaria vivax in Alusi community health center and 1 case in Waturu community health center. The presence of *P. vivax* cases indicated that the treatment was unfinished.

MBS activity results in the Kilmasa Village and Waturu Village can be seen in Table. From Table. 1 showed that of 923 samples slides of thick blood smear obtained 2 positive samples of *P. vivax*

Anopheles spp larvae surveillance was conducted in the Kelaan village and Kilmasa village. Observation results can be seen in Table 2. The type of mosquitoes that found from the result of colonization (rearing) in the field can be seen in Table 2. *Anopheles spp* species found in the study site i.e. *An. flavirostris*, *An. barbirostris group*, *An. farauti* and *An. koliensis*.

Habitat surveillance resulted that the mosquito *Anopheles spp* mostly found in water drainage, rain puddle, puddles on the boat/canoe, puddle on used car tires and puddle on used drums (Table 2). Habitat *Anopheles spp* larvae are generally exposed to direct sunlight, but there was also habitat of mosquito larvae that protected from direct sunlight. Exposure to light can directly affect dissolved oxygen levels that affect the development of mosquito larvae. However, some *Anopheles* species can survive in

Table 1. Mass Blood Survey (MBS) Results in Kilmasa Village and Waturu Village

MBS location	n sample	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. ovale</i>
Kilmasa village	489	0	1	0	0
Waturu village	434	0	1	0	0
Total	923	0	2	0	0

Source : Primary Data

Table 2. *Anopheles* spp Larvae Types Found on Several Waters in Alusi and Waturu Community Health Center, Maluku Tenggara Barat.

Surveillance location	Habitat type	<i>Anopheles</i> spp species found			
		<i>An. farauti</i>	<i>An. flavirostris</i>	<i>An. barbirostris</i> group	<i>An. koliensis</i>
Alusi village Kilmasa village	Water drainage	0	3	2	0
	Rain puddles	5	8	4	3
	Puddles on boat	5	0	0	0
	Puddles on used car tire	4	6	4	0
	Puddles on used drum	0	7	2	0
Waturu village	Water drainage	0	0	0	0
	Rain puddles	0	5	0	0
	Puddles on used boat	0	0	0	0

Source : Primary Data

these conditions (Kazwaini, 2015). The species of *Anopheles* spp were found in the study site, i.e. *An. farauti*, *An. flavirostris*, *An. koliensis*, and *An. barbirostris*. In addition, *Anopheles* spp larvae found in residential areas and in palm forest areas. Topography of study site was a beach area and close to the hilly area with dense palm forests. These topography characteristics was similar with the West Timor, where many *An. barbirostris* and *An. flavirostris* were found (Ndoen, 2010). Generally the habitat of *Anopheles* spp was formed as a result of human activities such as the bridges manufacture, drainage and road widening which form a hole that can form a puddle of water in the rainy season, a semi-permanent pool, puddle on used drums, irrigation canals that obstructed by wood and rubbish foliage. These places were also potential for habitat *Anopheles* spp (Elyasar, 2013).

From the detention resulted that *Anopheles* spp larvae density was quite low. *Anopheles* spp larvae density was said to be high if it is found 20 larvae per detention (Idram, 2000). Physical and environmental conditions in some types of marine habitats of *Anopheles* spp that found at the study site can be seen in Table 3. The physical and chemical characteristics were included habitat temperature of 27-28.3°C, 6-7 pH marine habitats with 0-1 ‰ salinity and 75-84% air humidity. Meanwhile, the biological environments were discovered vegetation of coconut palms, banana, mango, teak and grass, while aquatic vegetation such as kale, green algae and moss water. We found

some larval predators such as tin head fish and water spider. When we conducted the surveillance in residents, we did not found any adult mosquitoes *Anopheles* spp, however many *Anopheles* spp were found in the garden palm forest near the puddles and swamps. People who to do activity until evening to make copra and work in the garden until night had greater opportunity to get bite of *Anopheles* spp. Copra production activity also left coconut shell that may become rainwater reservoir for mosquito larvae habitat. According to Ruselland Santiago, flight distance of *An. Flavirostris* was 1-2 km from the larvae habitat (Sinka et al., 2011). *An. barbirostris* group has the ability to fly a maximum 1-1.2 km. Habitat of *Anopheles* spp larvae were found around the population indicated the magnitude of the risk of malaria transmission by mosquitoes adults *Anopheles* spp. The distance between the vector breeding habitats and human settlements is one of the risk factors of the malaria incidence (Taviv, 2015).

Table 3. Physical and enviromental conditions in some habitat type of *Anopheles* spp larvae in Alusi Primary Health Care in Kelaan village and Kilmasa village, Maluku Tenggara Barat Regency.

Entomology surveillance of adults *Anopheles* spp in Alusi community health center in the Kelaan village and Kilmasa village found *An. flavirostris*, *An. barbirostris* group, *An. farauti*, and *An. koliensis*. The surveillance was conducted using man landing collections inside and outside house. We found no adult females of

Table 3. Physical and Enviromental Conditions in Some Habitat Type of Anopheles spp Larvae in Alusi Primary Health Care in Kelaan village and Kilmasa village, Maluku Tenggara Barat Regency.

Physical and environmental conditions	Habitat types of <i>Anopheles</i> spp in <u>Alusi</u> Primary Health Care				Habitat types of <i>Anopheles</i> spp in <u>Waturu</u> Primary health care			
	Water drainage	Rain puddle	Puddles on the boat/canoe	Water reservoir made from used car tires	water reservoir made from used drums	Water drainage	Rain puddle	Puddles on the boat/canoe
Water temperature °C	27.6	28.0	28.1	28.3	28.1	26.6	27.0	28.1
pH	6	6	6	7	6	7	7	7.4
Air moisture (%)	80	75	78	83	84	80	75	78
Air temperature °F	85	78	78	85	83	85	78	78
Salinity	0	0	1	0	0	0	0	1
Depth (cm)	50	30	20 cm	20	50 cm	30	20 cm	20 cm
Bottom of waters	muddy	muddy	-	Soil sedimentation	Soil sedimentation	muddy	muddy	-
Water vegetation	<i>Ipomoea aquatica</i> , water moss, green Algae, <i>Eichornia crassipes</i>	<i>Ipomoea aquatica</i> , water moss, green Algae, <i>Eichornia crassipes</i>	-	-	-	<i>Ipomoea aquatica</i> , water moss, green Algae, <i>Eichornia crassipes</i>	<i>Ipomoea aquatica</i> , water moss, green Algae, <i>Eichornia crassipes</i>	-
Surrounding plants	<i>Imperata cylindrica</i> , <i>Cyperus rotundus</i> , banana plants, coconut palms	<i>Cyperus rotundus</i> , Coconut palm, mango trees, teak trees	-	-	-	<i>Imperata cylindrica</i> , <i>Cyperus rotundus</i>	<i>Cyperus rotundus</i> , Coconut palms	-
Plant shade	-	-	-	-	-	-	-	-
Plant density	dense	dense	residents	-	Sparse	sparse	sparse	Residents
Surrounding ecosystem	Bushes, coconut forests, residents	Bushes, coconut forests	-	Bushes, coconut forests	Coconut forests, teak trees	Coconut palms trees, residents	Bushes, coconut forests, residents	-
Water predator larvae	Laba-laba air, ikan kepala timah	Laba-laba air, ikan kepala timah	Laba-laba air	-	-	Water spiders,	Water spider, tin head fish	r
Distance from residents	50 meter	500 meter	100 meter	30 meter	5 meter	10 meter	20 meter	10 meter
Anopheles type	<i>An. barbirostris</i> , <i>An. flavirostris</i>	<i>An. Farauti</i> , <i>An. flavirostris</i> , <i>An. barbirostris</i> , <i>An. Kaliensis</i>	<i>An. farauti</i>	<i>An. farauti</i> , <i>An. kaliensis</i>	<i>An. barbirostris</i> group, <i>An. flavirostris</i>	-	<i>An. flavirostris</i>	-
Number of larvae	5	20	5	14	9	0	5	0
Larval density 50 detention (%)	10	40	10	28	18	0	10	0

Source : Primary Data

Anopheles spp at the residents of Kelaan village, however we found *Armigeres* sp, sp *Aedes* and *Culex* sp. Then, surveillance move to temporary residents near palm plantations where people usually making copra there. *Anopheles* spp surveillance in Kilmasa village obtained low density of *An. flavirostris* (28 mosquitoes *An. flavirostris*). In Kelaan village we found several species of *Anopheles* i.e. *An. flavirostris* (91%) and *An. barbirostris* (8.2%) with a relatively high

density. Entomology surveillance conducted in the Waturu community health center found *An. flavirostri* species with low density (2 mosquitoes *An. Flavirostris*), *Culex* and *Aedes* spp. The low surveillance result of *Anopheles* spp in the Kilmasa village and Waturu village caused by east wind that blowing hard along with heavy rain

Figure 1 showed the results of calculation of Man Biting Rate (MBR) for the species *An.*

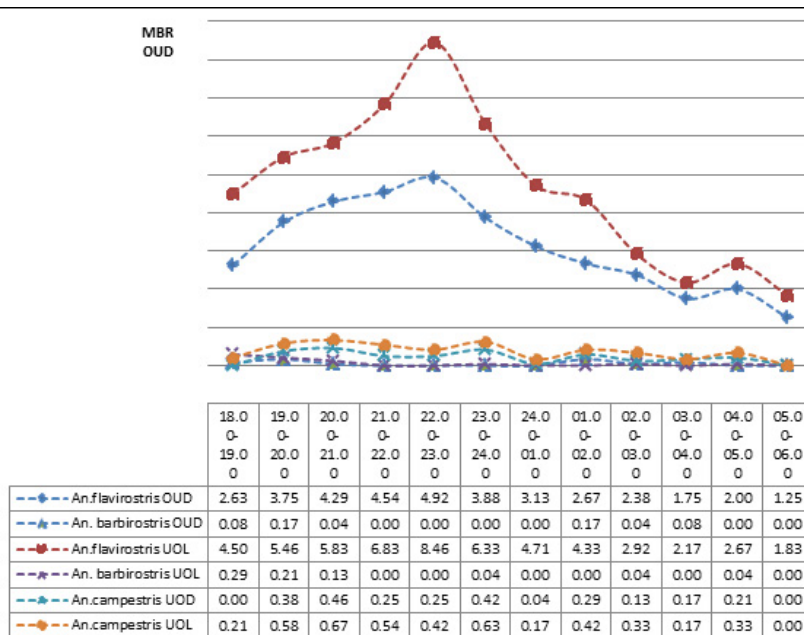


Figure 1 Mosquito Density of *Anopheles* spp Each Night

Table 4. Examination of Circum of *Plasmodium falciparum* Sporozoites 210 and *Plasmodium vivax* 210 and *Anopheles spp* from the Kelaan Village, Kilmasa Village and Waturu village, Maluku Tenggara Barat Regency

Location	Mosquitoes species	Total Sample	Sporozoites circum		Sporozoite Rate (%)
			<i>P.falciparum</i> 210	<i>P.vivax</i> 210	
Kelaan village	<i>An. Flavirostris</i>	1290	5	0	0,38
	<i>An.barbirostris</i>	32	3	1	12,5
	<i>An.campestris</i>	73	0	0	0
	<i>An. Farauti</i>	15	0	0	0
Kilmasa village	<i>An. Flavirostris</i>	28	0	0	0
Waturu village	<i>An. Flavirostris</i>	2	0	0	0

Source : Primary Data

flavirostris where the value MBR for OUD was 4.92 and OUL was 8.46. For MBR value of *An. Barbirostris*, the OUD was 0.05 and OUL was 0:06. For MBR value of *An. Campestris*, the OUD was 0.22 and OUL was 0.37, while the value of MBR *An. Farauti*, the OUD was 0.02 and OUL was 0.05. For MBR value of *An. Koliensis*, the OUD was 0 and OUL was $3,3 \times 10^{-3}$ (Figure 1).

The results of calculations Man Hour Density (MHD) of *Anopheles spp* in Kelaan Village. We found that MHD for catching mosquitoes *An.flavirostris* inside the house (OUD) was 0.79 and outside the house (OUL) was 1.37, whereas *An. koliensis* had a low density each hour, which for UOD the MHD value was 0 due to no mosquitoes were found and OUL obtained MHD values 8×10^{-4} persons/hour. *Anopheles spp* density was said high if the MBR is 1.0 per person-night, and the value of MHD 0.5 per person-hour (Idram, 2000). From these data, *An. flavirostris* and *An. barbirostris* had high biting activity inside and outside the house at night, so it is recommended that people should avoid *Anopheles spp* bite using netting or mosquito repellent before going to bed, close the windows and the door, reduce activities outside the house and put gauze on ventilation window. Besides that, people who often overnight/stay in the garden were also advised to bring mosquito nets and wear clothing to avoid mosquito bites (Dale et al., 2005).

Research (Boesri, 2006) in Magelang District reported that *An. flavirostris* and

An. barbirostris active biting inside the house, outdoors and in salak farms. Research concerning on the biting activity of *An. flavirostris* in East Flores found that the mosquito actively bite throughout the night and peaked at 09:00 pm to 1:00 am (Barodji, 1999, Boewono, 2005; Boewono, 2012). This contrasts with the behaviour of biting of *An. flavirostris* in Donggala where density peaks at 07:00 pm to 09:00 pm, while for *An. barbirostris* peak biting density at 12.00 pm to 01.00 am. This indicated that the biting behaviour vary for each area where mosquitoes *Anopheles spp* inhabited (Jastal, 2007). In this study, we also found low density of *An. farauti* and *An. Koliensis*. These mosquitoes were common in Papua and were vectors of malaria and filariasis (Sinka, 2011; Elyazar, 2013).

Table 4 showed the results of an examination of the circum sporozoites *An. flavirostris*, which 5 mosquitoes found positive had sporozoites of *P. falciparum* 210 but no sporozoites of *P. vivax* 247 found. We found three *An. Barbirostris* mosquitoes were positive of *P. falciparum* 210 and one positive of *P. vivax* 210. *Anopheles flavirostris* which found positive of sporozoite *P. falciparum* actively biting at 11.00 pm-12.00 pm. *Anopheles barbirostris* which found positive sporozoite *P. falciparum* and *P. vivax* actively bite at 11.00pm-12.00pm. Research in several locations in Indonesia found that *An. flavirostris* and *An. barbirostris* is confirmed as malaria vector using an examination of the circum sporoit by ELISA

Tabel 5. Examination Results of Blood feed *Anopheles spp* Using Techniques Enzyme Linked Immunosorbent Assay (ELISA)

Mosquito species	Total Abdomen Sample	Mosquito	Positive Sucking	Human Blood	HBI (%)
<i>An. flavirostris</i>	15		5		33.3
<i>An. barbirostris</i>	8		6		75
<i>An. campestris</i>	4		2		50

Source : Primary Data

method in East Nusa Tenggara, West Nusa Tenggara, North Sulawesi, Southeast Sulawesi and Gorontalo (Dale., 2005; Ndoen, 2010; Elyazar, 2013). The results of sporozoite rate for each study examined was differences caused by difference of the number of mosquitoes and also inspection method used, for *An. barbirostris* amounted to 0.95% (91/9568) and *An. flavirostris* 0.09% (2/2175) (Elyazar, 2013).

Table 5 showed the results of blood feed samples using mosquito *Anopheles spp* were resting in nature such as below a teak tree leaves, grass and tree trunk. Examination results by ELISA for human blood index (HBI) were 33.3% for *An. flavirostris* and 75% for *An. barbirostris*. Research conducted in Sulawesi Tengah obtained the HBI was 28.6% for *An. barbirostris* and 9.1% for *An. flavirostris* (Elyazar, 2013).

This study is still basic research which has many limitations due to the properties of the study where the only assessed on the spot. For future needs, we recommend to perform entomology longitudinal surveillance to see monthly entomology parameters in a full year and the influence of the environment (climate and weather) in describing the transmission of malaria whether to be highly endemic and stable in Maluku Tenggara Barat (Dale, 2005).

Conclusion

The proportion of malaria cases when performed Mass Blood Survey (MBS) in the Kilmasa village and Waturu village were 0.20% and 0.23%, respectively. Entomology surveillance of *Anopheles spp* species found in Maluku Tenggara Barat Regency was *An. flavirostris*, *An. barbirostris* group (*An. barbirostris* and *An. campestris*), *An. farauti* and *An. koliensis*. Habitats of *Anopheles spp* were semi-permanent pools, puddle on car tires, drum, and used boat, water drainage, and pools.

Anopheles spp active biting at 06:00 p.m. to 06:00 am with the highest mosquito density at 10.00 pm to 11.00 pm. Vector confirmation results using Enzyme Immunoabsorbent Linked Assay (ELISA) found that *An. flavirostris* positive containing *P. falciparum* sporozoites circum 210 and *An. barbirostris* positive containing *P. vivax* sporozoites circum 210 and *P. falciparum* 210. We recommend intervention that may reduce malaria mosquito larvae breeding sites such as hoarding holes road construction, used drums, waste coconut copra and unused boat was reversed or destroyed to minimize mosquito larvae habitat in the rainy season. For early detection of parasite malaria, the health workers should actively doing active case detection (ACD) and passive case detection (PCD) by performing mass fever survey (MFS) and the mass blood survey (MBS) and provide treatment as soon as possible on positive cases of malaria so that prevent transmission from patients to mosquitoes, hence the life cycle of the parasite may be stopped. We encourage to do community development toward malaria control to minimize mosquito breeding places through improved sanitation, environmental modification, use of mosquito nets containing insecticide and reduction of population.

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