

The malaria vectors of *Anopheles flavirostris* and *Anopheles barbirostris* in West Southeast Maluku Regency

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Received 22 November 2014; received in revised form 17 January 2015; accepted 28 February 2015

Abstract. Malaria is one of the health problems in West Southeast Maluku Regency, with Annual Parasite Incidence (API) is 15%. There are not many surveys to studies conducted on vectors of malaria. This research aims to know the habitat, species of *Anopheles* sp., and know the malaria vector species in West Southeast Maluku area. This research was conducted between March – May 2016 in Alusi and Waturu villages. The method of collection was by man landing collections and examination on circum sporozoite protein using enzyme-linked immunosorbent assay (ELISA) technique. Result of research was obtained the samples of *An. flavirostris* is 2 265 mosquitoes and *An. barbirostris* is 32 mosquitoes, with the biting activity was found to reach its peak at 19.00p.m-23.00 p.m. having characteristics of exophilic, exophagic and anthropophilic. The life expectancy of *Anopheles* sp. in nature is 62-77% with the life span of *Anopheles* sp. in the nature is below 2-4 days. *Anopheles flavirostris* and *An. barbirostris* positively contain the circum sporozoite protein (CSP) of *Plasmodium falciparum* and *Plasmodium vivax*.

INTRODUCTION

World health organization reported a number of malaria case in 2015 predicted to be 214 million, where 80% was in Africa, 10% was in Southeast Asia, 2% was in East part of Mediterranean. Meanwhile, the number of death caused by malaria in 2015 was predicted to be 438 000 where 90% was in Africa, 7% was in Southeast Asia and 2% was in east part of Mediterranean (WHO, 2015). In Indonesia, the case of malaria is predicted to be 15 million of cases with the case of death is 42 000 per year. This disease is caused by *Plasmodium* sp. (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) and transmitted by the bites of malaria vector *Anopheles* sp. Mosquitos in Indonesia which have been reported namely *An. balabaciensis*, *An. sundaicus*, *An. manculatus*, *An. subpictus*, *An. punctulatus*, *An. koliensis*, *An. barbirostris*, *An. acunitus* and *An. farauti* (Ndoen *et al.*, 2010)(Ndoen *et al.*, 2012).

West Southeast Maluku Regency is one of the malaria endemic areas in Maluku Province with the number of Annual Parasite Incidence (API) is 15%. The data regarding the malaria vector in West Southeast Maluku regency is not greatly published. The entomology survey conducted by local Health Office mentions that there are some *Anopheles* sp. namely: *An. punctulatus*, *An. koliensis*, *An. farauti* and *An. tessellatus*. Maluku Province is Indonesia East Region in the Weber line so that there are quite high diversities of *Anopheles* sp., the malaria vectors in Indonesian East Region are *An. punctulatus*, *An. koliensis*, *An. farauti*, *An. bancrofti*, *An. subpictus* and *An. barbirostris* (Munif *et al.*, 2008). The availability of basic data regarding the malaria vector bio-ecology in this area can be used as the vector control intervention program so that the number of malaria morbidity can be decreased (Krismawati *et al.*, 2015).

MATERIALS AND METHODS

Research Area

The research was conducted between March – May 2016 in Alusi and Waturu village, West Southeast Maluku Regency (Kabupaten Maluku Tenggara Barat) in Indonesia. The geographical location of Maluku is 06°–08°30' of South latitude and 120°45'–133° East longitude. The West Southeast Maluku Regency is a island area, low land, hills and mountain area. The season in this area is east Monsoon season between April–September (Dry Season) and west Monsoon season between October–March (Rainy Season) with relatively high rainfall. There is the transition season between March–April and September–October. The Alusi village lies in the middle part of the District of Komormolin Tanimbar islands south (geographical location is 7°43'45.84" South latitude, and 131°31'45.58" East longitude) and Waturu village including District of Tutukembong located in the eastern part of the island Tanimbarese south (geographical locations is 7°37'45.30" South latitude and 131°37'34.71" East longitude. Alusi and waturu villages an area near the beach with a height of about 500 meters. It has a forest vegetation of coconut trees and shrubs.

Field Data Collection

This study was approved by the research ethics of the National Institute of Health Research and Development (Ethics Approval Letter Number: LB.02.01/5.2/KE.157/2015). A survey on catching adult *Anopheles* sp. mosquitoes along the night was using based on a technique from WHO, namely man landing collections where the catch was conducted starting at 18:00 p.m to 6:00 a.m, the survey was conducted in 3 houses and with the number catcher of 4 people, two people were in the house and two people were outside the home. The catch duration was 45 minutes with a 15 minute break. A catching on the mosquitoes resting in nature was done at 06:00 a.m to 8:00 a.m by 2 people for 30 minutes. In the research area, it was not conducted the catch at the cattle shed because local people did not have any cattle living in the sheds. The *Anopheles* sp.

mosquitoes caught later were identified their morphology using a stereo microscope (Nikon) and the identification of key book morphology of *Anopheles* sp. in Indonesia region (Assem & Wepster 1959)(Peyton & Scalon 1966)(Reid 1968)(O'Connor & Soepanto 1999)(Department of Health, 2000).

Determination of malaria vector potential on the *Anopheles* sp. mosquito obtained in the research area was conducted by detecting the circum sporozoite protein (CSP) of *Plasmodium* sp. on the head-thorax part of the mosquitoes using an enzyme-linked immunosorbent assay (ELISA) technique. The *Anopheles* sp. which have been identified were segregated their head-thorax and abdomen, then the head-thorax were inputted in 1.5 mL of eppendorf microtube containing 50 µL of blocking buffer (KPL Cat. No. 50-61-01) and 50 µL of NP-40 and ground with pastel pellets. The samples were then rinsed with blocking buffer to a total volume of 200 µL, the samples were stored overnight at 4°C. Then, each microplate (IWAKI Pyrex Cat. No. 3801-096) was coated by capture monoclonal antibody *P. falciparum* 210 (KPL Cat. No. 37-00-24-2) 0.2 µg / 50 µL of PBS (Gibco™ PBS Buffer Cat. No. 70011044) and other microplate was coated by capture monoclonal antibody *P. vivax* (KPL Cat. No. 37-00-24-2) with 0.025 µg / 50 µL of PBS then incubated for 1 hour. Then, the capture monoclonal antibody was aspirated then added by 200 µL of blocking buffer and incubated for 1 hour. Blocking buffer on wells is aspirated then put 50 µL of mosquito samples, 50 µL of negative control (colonization of *Anopheles* sp. in the laboratory) and 50 µL of positive control *P. vivax* and *P. falciparum*, then it was incubated for 2 hours. The samples were aspirated and washed with 200 µL of PBS-0.05% Tween-20 twice. Each microplate well is added by 50 µL of peroxidase monoclonal antibody *P. falciparum* 210 (KPL Cat. No. 37-00-24-3) and the other plate was by 50 µL of peroxidase monoclonal antibody *P. vivax* 210 (KPL Cat. No. 37-00-24-3), then incubated for 1 hour. Conjugate peroxidase monoclonal antibody was aspirated and then washed with 200 µL of PBS-0.05% Tween-20 for 3 times. It was added by 100 µL substrate ABTS (KPL

Cat. No. 50-66-18), there would be color change for 30-60 minutes. For the examination of blood meal mosquitoes, it was conducted by ELISA with almost the same technique on the examination of sporozoites in head-thorax mosquitoes (Ferreira *et al.*, 1986)(CDC 2007)(Barbara *et al.*, 2011).

RESULT

The research was conducted between March –May 2015 in Alusi and Waturu Villages. The number of sample of *An. flavirostris* mosquitoes found in Alusi village for the catch inside the houses (outdoor) was 900 and the catch outside was 1 365 (in door). Meanwhile, the number of sample of *An. barbirostris* mosquitoes inside the houses (indoor) was 14 and outside (outdoor) was 18. The catch of *Anopheles* sp. in Waturu village was obtained 2 mosquitoes. This was caused by quite high rainfall and sea breeze was blowing quite hard. The morphology of *An. flavirostris* and *An. barbirostris* we can show in Figure 2 and Figure 3. The man biting rate per night can be seen in Figure 4. The biting activity of *An. flavirostris* mosquito is started at 18.00 p.m and reaches its peak

at 22.00 p.m – 23.00 p.m. The *An. flavirostris* actively bites along the night until 05.00 a.m in early day. Meanwhile, the *An. barbirostris* actively bites starting at 18.00 p.m and reaches its peak at 20.00 p.m and 01.00 a.m in early day. This mosquito is also found to actively bite along the night until 05.00 a.m in early day (can be seen in Figure 4).

The man hours density of *An. flavirostris* per night reaches its peak at 22.00 p.m – 23.00 p.m mean while the man hours density of *An. barbirostris* per night starts at 19.00 and 01.00 in early day (can be seen in Figure 5).

Table 1 shows the results of the sporozoite examination using ELISA technique on the *An. flavirostris* showing positive *P. falciparum* with sporozoite rate of 0,38% mean while, the examination on the sporozoite of *Plasmodium* sp. in *An. barbirostris* is found to be positive on the *P. falciparum* and *P. vivax* with the sporozoite rate of 12.5%.

The examination of blood meal in abdomen of *Anopheles* sp. used the mosquitoes resting in the nature. The catch was conducted for 1 hour at 06.00 a.m–07.00 a.m. Table 2 shows the result of examination of blood meal of *An. flavirostris* from the examination using ELISA technique, it is

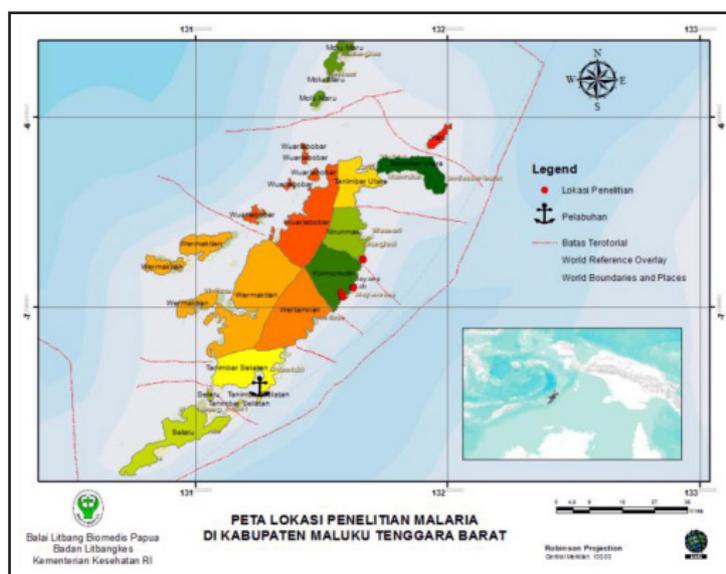


Figure 1. Research location map of *Anopheles* sp. sample collection (red dots) in West Southeast Maluku Regency, Maluku Province.



Figure 2. Morphology of *Anopheles flavirostris*.



Figure 3. Morphology of *Anopheles barbirostris*.

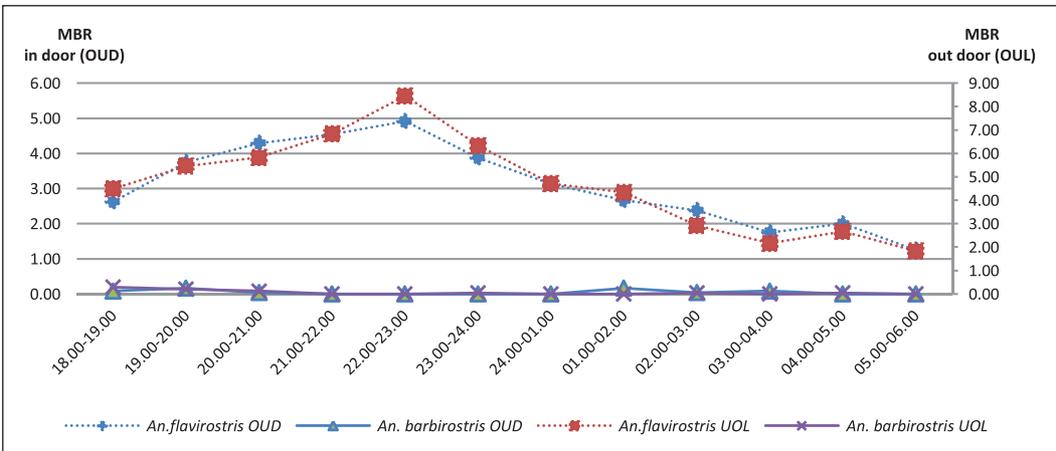


Figure 4. Man biting rate *An. flavirostris* and *An. barbirostris* in West Southeast Maluku Regency.

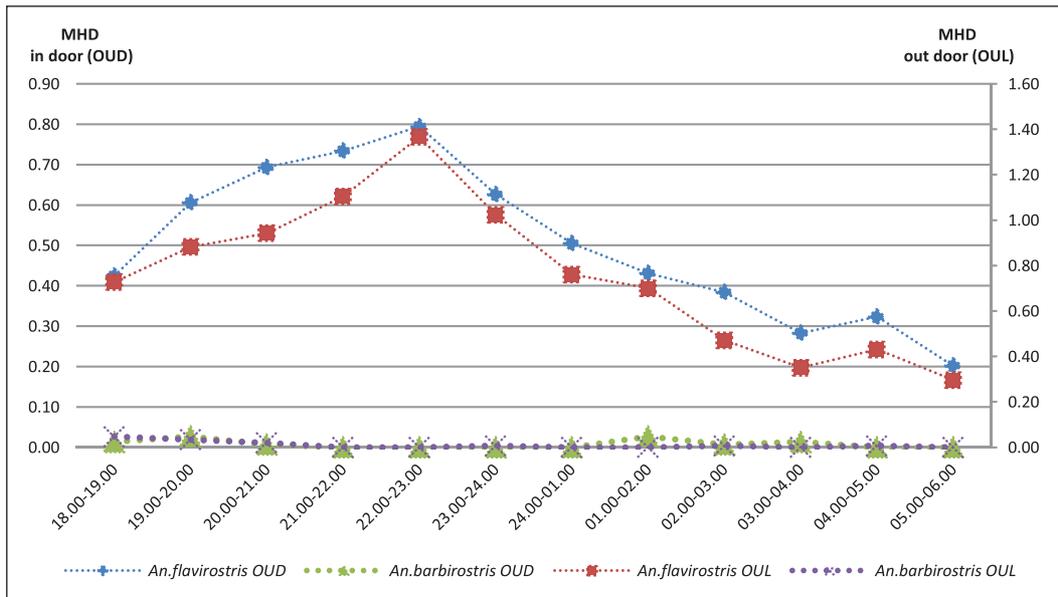


Figure 5. Man hours density *An. flavirostris* and *An. barbirostris* in West Southeast Maluku Regency.

Table 1. Results of examination of sporozoite *Plasmodium falciparum* and *Plasmodium vivax* in *Anopheles* sp.

Location	Species of mosquito	Number of sample	Circum sporozoit		Sporozoite Rate (%)
			<i>P. falciparum</i> 210	<i>P. vivax</i> 210	
Kelaan Village	<i>An. flavirostris</i>	1290	5	0	0.39
	<i>An. barbirostris</i>	32	3	1	12.5
Kilmasa Village	<i>An. flavirostris</i>	28	0	0	0
Waturu Village	<i>An. flavirostris</i>	2	0	0	0

Table 2. Human blood index (HBI) *An. flavirostris* and *An. barbirostris*

Speccies of mosquitoes	Number of sample mosquito abdomen	Positively sucking human blood	HBI (%)
<i>An. flavirostris</i>	15	5	33.3
<i>An. barbirostris</i>	8	6	75

obtained the human blood index (HBI) of 33.33% meanwhile for the *An. barbirostris*, it is obtained the HBI of 75%.

The determination of mosquito parous rate and age in nature was conducted by using the Devidson equation. Table 3 Calculation

result of parous rate and age of *An. flavirostris* and *An. barbirostris* in the nature. In this study using genotropic cycle for three days to calculate life expectancy and life span for *An. flavirostris* and *An. barbirostris*. It is based on the cycles

Table 3. Age of *An. flavirostris* and *An. barbirostris* in West Southeast Maluku Regency

Species of mosquitoes	Number of dissected mosquito	Parous (P)	Parous rate (PR)	P = $\frac{gc}{\sqrt{PR}}$	Age (Days) Mosquito = $\frac{1}{-lnP}$
<i>An. flavirostris</i>	50	23	0,46	0,77	3,83
<i>An. barbirostris</i>	19	5	0.26	0,63	2.16

genotropic on Malaria vectors *An. leucosphyrus* group for 3 days (Ndoen *et al.*, 2012).

DISCUSSION

The entomology survey of *Anopheles* sp. in Alusi and Waturu villages found the *An. flavirostris*, *An. barbirostris*, *An. campestris*, *An. koliensis*, *An. farauti*. The species greatly found were *An. flavirostris* and *An. barbirostris*. The habitats of *Anopheles* sp. larvae found were semi-permanent pool, drum and tire as place for water storage, puddle at the damaged boat. While, the vegetation were grass, water spinach, mango and coconut trees. Alusi and Waturu village is located near the coast (The distance is ± 500 meter from costal), but at the time of the survey the adult mosquito in villages are not found of *Anopheles* sp. This is due to the rainy weather and the wind is blowing hard. Survey location in Alusi Villages was moved to the palm plantation forest (The distance is ± 1 Km from costal and The elevation is ± 950 meters) with the vegetation of coconut palm trees, mango trees, teak trees, grasses, and shrubs. This location is found many species of *Anopheles minimus* complex (*An. flavirostris*), *Anopheles barbirostris* group (*An. barbirostris* and *An. campestris*) and *Anopheles punctulatus* group (*An. farauti*, *An. punctulatus* and *An. koliensis*). Total population of adult *Anopheles* sp. mosquito that many common, namely *An. flavirostris* and *An. barbirostris*. Survey of adult mosquitoes in the Waturu (The distance is ± 50 meter and The elevation is ± 15 meter) villages discovered species *Anopheles flavirostris* with a population of slightly due

to strong winds accompanied by heavy rain. Other research the existence of malaria vector of *An. barbirostris* in Indonesia spreads in Sumatra, Sulawesi, Java, Sunda, Bali, Kalimantan, Maluku Island, and Timor-Leste Island. *Anopheles flavirostris* is mostly found in South Sulawesi, Java, Sunda, Timor-Leste Island (Sinka *et al.*, 2011)(Elyazar *et al.*, 2013). West Southeast Maluku Regency is the administrative area of Maluku Province. In this location, there is no report on many researches concerning the existence of *Anopheles* sp., the surveys conducted by Provincial Health Office concerning malaria; the most found vector are *An. punctulatus*, *An. koliensis*, *An. farauti* around Saumlaki and Olilin. These species are mostly found in Papua region while, the *An. flavirostris* and *An. barbirostris* are mostly found in West part of Indonesia. The location of Southeast Maluku Regency is in Weber line as the meeting of species from West region of Indonesia and East region of Indonesia. The existence of *An. flavirostris* vector is not many reported. This vector is rarely found in paddy field area. The *Anopheles flavirostris* is found at the height of 600 m -1 500 m, commonly in coastal area; it is found the larvae habitat in slow water flow, dense florest vegetation with grass (Sinka *et al.*, 2011). *Anopheles flavirostris* is mostly found to rest in zallaca leaf litter and cliff of zallaca garden. It is also reported that it takes rest on the ground holes in the edge of river (Alfiah *et al.*, 2008). In the research area, the *An. flavirostris* is found to rest under the teak tree leaves, coconut tree and grass.

The man biting rate of *An. flavirostris* outside the house is 8,46 people per night and inside the house is 4.92 people per night, so that the mosquito has the characteristic of

exophagic. In the research area, the *An. flavirostris* actively bites and reaches its peak at 22.00 p.m-23.00 p.m, this is not much different to some reseacrh mentioning that the *An. flavirostris* bites along the night, (Sinka *et al.*, 2011). This is different to the man biting rate by *An. flavirostris* in East Timur at 19.00 p.m-05.00 a.m (Barodji *et al.*, 1999). The man biting rate of *An. flavirostris* outside the house is 1.37 people per hour and inside the house is 0.73 people per night so that it has the characteristic of exophagic. This mosquito is more active to bite outside the house than inside the house.

The habitat of *An. barbirostris* larvae is reported to be mostly found in marshes, fish pond, paddy-fields, water drainage, lagoon, former puddle and car / cart and water springs (Boesri & Boewono 2006)(Jastal *et al.*, 2007)(Rahmawati *et al.*, 2014). The habitat of *An. barbirostris* larvae is in Alusi village such as in water drainage, puddle, water storage from drum.

The man biting rate (MBR) per night of *An. barbirostris* outside the house is 0.13 and inside the house is 0.04 so that the mosquito has the characteristics of exophagic. This result is not much different to a research by Noshirma *et al.* (2011) in Sumba regency, East Nusa Tenggara which the MBR outside the house is 0.08 and MBR inside the house is 0.04. In the research area, *An. barbirostris* actively bites at 19.00 p.m-20.00 p.m. This research is accordance with a research by Limrat *et al.* (2001) and Munif *et al.* (2008) reporting that the peak of biting activity by *An. barbirostris* starts at 19.00 p.m-20.00 p.m (Limrat *et al.*, 2001)(Munif *et al.*, 2008). This is different to a research by Donggala mentioning that the peak of biting by *An. barbirostris* is at 21.00 p.m-03.00 a.m (Widjaya *et al.*, 2006)(Elyazar *et al.*, 2013)(Noshirma *et al.*, 2012). So, the geographical and environmental differences affect on the biting activity behaviour by *Anopheles* sp. (Munif *et al.*, 2008). The man bite rate per hour by *An. barbirostris* outside the house is 0.034 and inside the house is 0.027 so that the mosquito has the characteristics of exophagic.

The examination of sporozoite at head-thorax part of *An. flavirostris* and *An.*

barbirostris by ELISA technique is to detect the antigen circum sporozoite (CSP) of *P. falciparum* 210 and *P. vivax* 210. The examination result of *An. flavirostris* is found that *P. falciparum* 210 positively with sporozoite rate (SR) of 0.39% while, the *An. barbirostris* is found positively of *P. falciparum* 210 and *P. vivax* 210 with Sporozoite rate (SR) of 12.5%. *Anopheles flavirostris* reported is the malaria vector in Sulawesi, while the *An. barbirostris* is also reported to be the malaria vector in Sulawesi, Java, Bali, Lampung, and Seribu Island (Munif *et al.*, 2008)(Ndoen *et al.*, 2010). The examination of blood meal of *An. flavirostris* is obtained that the human blood index (HBI) is 33.33% while, for the *An. barbirostris*, it is obtained the HBI of 70%. This shows that the *An. barbirostris* has the characteristic of antrophophilic than the *An. flavirostris*. Although, it is necessary to be wary on the existence of *An. flavirostris* which can have the potential as malaria vector in Alusi and Waturu villages. The research location is not found livestock such as cattle, goats, and buffaloes that feed blood meal in abdomen of *Anopheles* sp. more dominant containing human blood.

The age determination of mosquito in the nature is done by dissecting the ovary of female *Anopheles* sp. The Parous rate of *An. flavirostris* is 0.46 (46%), the life expectancy in nature is 0.77 (77%) and life span of *An. flavirostris* can reach 3.83 days while, the parous rate of *An. barbirostris* is 0.26 (26%), the life expectancy in nature is 0.64 (64%) and life span of *An. barbirostris* can reach 2.16 days. The parous rate and life span of mosquito determines whether the mosquito can be the malaria vector (Mardiana & Munif 2009). The longer the life span of *Anopheles* sp. lead the greater chance to be the malaria vector because the parasite of *Plasmodium* sp. can settle its life cycle to be sporozoite and reach the saliva gland (Boewono *et al.*, 2012). The mean life span duration of *Anopheles* sp. in nature can reach 10-14 days and it can usually reaches 21 days. In the laboratory condition, life span of *An. barbirostris* can reach 34 days. In this research, it is found life span of *Anopheles* sp. below 4 days, this described that there

may be no longer any *Anopheles* sp. with long life. It is because of the malaria control program by using long lasting insecticide nets (LLINs) and the provision of Larvicide, showing that the caught one is still young *Anopheles* sp. (Ndoen *et al.*, 2012). Limitations of the study conducted using the method of microscopically to identification of mosquito morphology and using key book identification some times cannot description of complex species, and sporozoites detection using ELISA method that has many disadvantages because give positive false and cross reactions (Durnez *et al.*, 2011). In the future there will be identification detection for the determination of molecular species of *Anopheles* sp. and malaria vector using polymerase chain reactions (PCR) and sequencing. From the research results, it can be concluded that the malaria vectors found in West Southeast Maluku Regency are *An. flavirostris*, *An. barbirostris*, *An. campestris*, *An. koliensis* and *An. farauti*. *Anopheles flavirostris* and *An. barbirostris* have quite high species density level from other *Anopheles* sp. The man bite rate of *Anopheles* sp. reaches its peak at 19.00 p.m–23.00 p.m; it has the characteristics of exophagic, exophilic and anthropophilic. The conformation results of vector by technique gives the positive result of circum sporozoite protein of *P. falciparum* and *P. vivax*.

Acknowledgment. The gratitude is given to The Head of Health Research and Development hall, Minister of Health, Head of Biomedic Health Research and Development hall of Papua, Drh Rita Marleta Dewi, M. Kes, Head of Health Office of Maluku Province, Head of Health Office of West Southeast Maluku Regency, Head of Community Health Center and the Health officers in Waturu and Alusi villages.

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