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Genetic Diversity of Anopheles sp as Malaria Vectors Who Carries Plasmodium Falciparum and Plasmodiumvivax Which Can Infect Human in Jayapura Municipal, Papua Province, Indonesia

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ABSTRACT

Background. Jayapura municipal are region which are rainfall not erratically down will affect the development larvae of *Anopheles* mosquito which are can be threat of outbreak still remains due to the high prevalence and abundance on malaria vectors. Aim. The aim of this study to analyze species of *Anopheles* mosquito and genetic diversity species of *Plasmodium* which are found into *Anopheles sp* as malaria vector which are cause malaria in Jayapura Municipal. Methods. This type of research is a descriptive study using a cross sectional design. Adult of mosquito Anopheles were collected from four study sites located in in two district using human landing catch and aspirators. Representative samples of each species which are morphologically confirmed were selected of each locality in generally was found there is higher areas and low areas. Results. A total of 38 samples from *Anopheles sp* which are found by determination key shown that *An. punctulatus* as mush as 23(60.5%), *An. koliensis* 13 (34.2%) and *An. farauti* 2(5.3%) respectively with Pv < 0.05, and analyze through DNA extracted by PCR product, we did not found DNA bands from *P. falciparum* and *P.* vivax. Conclusions. The result of this study shown which are Pv < 0.05, there were significant correlation between located with Anopheles sp. Genetic diversity of *Anopheles* sp based on PCR product, overall not found DNA bands of *P. falciparum and P.vivax* because probably *Anopheles* mosquito species which such the blood of the captured person has not been infected by the both *Plasmodium* above in Hamadi rawah areas.

Keywords: *Genetic diversity, Anopheles sp, P. falciparum, P. vivax, Malaria.*

Introduction

Malaria is still remain a major public health of morbidity and mortality with a concerning issue of increase incases that reported in the 2017.According to thereport there were 212 million new cases of malaria worldwide in 2015. The incidence become 148-304 million clinical cases of malaria each year, and most them are caused by *P. falciparum* and *P. vivax*[1,2]. The report draws on data from 91 countries and regions with ongoing malaria transmission in 2016[3].

Malaria is an infectious disease caused by protozoan parasites from the Plasmodium family which can be transmitted by bitten of the *Anopheles* mosquito. Falciparum malaria is the most deadly type. The symptoms of malaria include cycles of chills, fever, sweats, muscle aches and headache that recur every few days. There can also be vomiting, diarrhea, coughing, and yellowing (jaundice) of the skin and eyes. Persons with severe falciparum malaria can develop bleeding problems, shock, kidney and liver failure, central nervous system problems, coma, and die[4].

Its epidemiology is determined by three components; the human host, the *Plasmodium* malaria parasite, and

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the environment, The latter includes both the psysical and biological environment is female mosquito Anopheles as malaria vectors[5]. The majority of mosquito in Papua Province are *An. farauti*, *An. Punctulatus* and *An bancrofti* whereas *An,koliensis* and *An. Kowari* are secondary vectors[4,6].

In Indonesia, the populations were living in endemic areas of malaria, local transmission is still at risk of malaria. By 2014 there are 74% of the population living in malaria without risk areas and 3% living in high risk areas. In the last 4 years most of the population lives in free Malaria areas whereas the population shows an increasingly. While people in high endemic areas have the lowest pretentage and level to fall from 4.7% in 2012 to 2.2% in 2015[5].

In Papua Province of Indonesia, malaria is a major health problem because this area is one of malaria endemic areas with hyper-endemic category in Indonesia.. Regency Health office reported in Jayapura that the annual parasite incidence (API) of malaria cases in 2014 was 90 per 1000 populations and Annual Malaria Incidence (AMI) is still 122 per 1000 populations[7].

Clinical Manifestation of malaria is influenced by several factors in the human host, *Anopheles* sp, parasite and enviroment. In human, age, immunity, pregnancy and genetic factors have been shown to determine the malaria cilinical outcome whereas in the malarial parasite, drug resistance, multiplication rate, invansion pathway, cytoadherence and rosetting, antigenic variation and polymorphisms, and malaria toxin are among other factors that have been identified[8].The mosquito species as malaria vectors mainly in Indonesia, expecially in Papua are *An, punctulatus, An. farauti,*and *An koliensis, respectively* which can be cause falciparum malaria, vivax malaria, and malariae malaria however ovale malaria rarely found in Papua, Indonesia[4,6].

Genetic diversity of *Anopheles* sp has the effect of the capacity value occurrence transmission and the vector's ability to transmit malaria[9,10]. Understanding the biology and behavior of *Anopheles* sp could be help understood how malaria is transmitted and can aid designing appropriate control strategies. Factors that effect a mosquito's ability to transmit malaria include it's innate susceptibility to *Plasmodium*, its host choice and its longevity[11], and difference in habitat conditions and community environment will be also affect the distribution of *Anopheles* in one area.

Material and Method

Description Study Site: This study was conducted in September to November 2017.Mosquito samples are obtained from different locations in Jayapura Municipal namely, Hamadi rawah, Skyline and Organda villages. In the microscopic examination, we were conducted at Laboratory of polytechnic of Health, Ministry of Health Jayapura and Eijkman Institute laboratory in Jakarta. Jayapura Municipal its wide territory covering 442,540 km^2.Jayapura Municipal is divided into mainland, swamp (146,576 ha), river area and large heading to the Pacific Ocean. The Municipal is bordered in the North through Pacific Ocean and in the east with Papua New Guinea. The populations of Jayapura Municipality is mainly Papuan, migrants Java, Sulawesi, Moluccas and the other parts of Indonesia.

Hamadi rawah village, this place is a lot of *Mangrove* trees which are a breeding ground for larvae of *Anopheles* sp. People were living in these areas from Papuan and non-Papuans tribes with a high population density the same with skyline and Organda villages[12,13]

The climate is typically tropical with average temperature between 25-35^{o}C. The difference between rainy season and dry season as because of wind effect. May to November, the wind is blowing from South east with less amount of water vapor whereas in December to April the westerly wind is blowing sea and causes rainfall. The range of rainfall is between 1,500-6000 mm per year[6].

Mosquito collection and identification: There are several sites in Jayapura Municipal which we were collected sample of mosquitoes. The technic for obtain sample we use human landing catch method. The mosquitoes that select reside around the resident's house by using aspirator. After sample we collect and inserted into the paper cup and then covered with gauze, on top of which was placed cotton which had been fed mosquitoes to keep the mosquitoes alive until identified process in the laboratory. Collecting malaria vectors we start from 06.00 pm to 06.00 am with long catch for 15 minutes with an interval of 1 hour[12,13]. For identification we were using determination key in Polytechnic Health Laboratory in Jayapura and molecular laboratory of Malaria Eijkman Institute in Jakarta.

Mosquito DNA Extraction: Individual mosquitoes were crushed in 1.5 mL micro-centrifuge tubes (Eppendorf, Hamburg, Germany) containing 100 µL of

lysis buffer (0.2 M NaCl, 10 mM Tris HCl pH 8,25 mM EDTA, 0.5% sodium dodecyl sulfate) containing1.0 mg/ml of proteinase K and then incubated at 55°C for 2 hour prior to being extracted twice with 50 µL of chloroform: iso-amyl alcohol (24:1). The upper aqueous layer was transferred to a new tube and the DNA was precipitated by adding 50 µL of 7.5 M ammonium acetate and 300 µL of ice cold absolute ethanol. The tubes were then placed at -70 °C. for 15 min, microfuge at 4°C for 15 min, and then washed in 500 µl of ice cold 70% ethanol. The pellet was dried and reconstituted in 50 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing RNase (100 µg/ml) [14,15,16].

Primer Selection and Design: The primer designed ITS2A was designed as a 19-mer from the 5.8S rDNA of Drosophila melanogaster (5'TGTGAACT GCAGGAC A CAT) and the primer ITS2B was designed from common invertebrate sequences at the 5' end of the 28S rDNA (5' TATGCTTAA ATT CAGG GGGT). The oligonucleotide primers were constructed on an applied. Biosystems (Foster City, CA) 394 DNA/RNA Synthesizer[14,15,16].

Amplication of ITS2: All PCRs were carried out in 0.5ml microfuge tubes in a 25 µl volume using a Minicycler PTC-150 (MJ Research Inc. Watertown, MA). The final PCR mixture contained 50 mM KCl, 10 mM Tris HCl pH 9.0.1.5% Triton X-100,1.0 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 50 pM of each primer, 10% Dimethylsulfoxide (DMSO),and 2.5 units of *Taq polymerase*. The template was either purified DNA (1-10 ng), 1µl of allozyme triturate (reconstituted in 20 µl of double distilled water) or from a single leg placed in the PCR. Cycling involved an initial denaturation at 94°C for 5 min prior to the addition of *Taq enzyme* and an oil overlay and then 35 cycles at 94°C for 1 min, 51°C for 1 min, and 72°C for 2 min using minimum transition times[16,17].

Product digestion and visualization: A 5µl aliquot of the PCR mixture was added to water, 2.5µl of 10 x *msp1* buffer and 10 x bovine serum albumin (10 mg/ml) and 1µl of *msp1* restriction endonuclease (20 units: New England Bio labs, Beverly, MA) to give a total volume of 20µl, and the sample was incubate at 37°C for 2 hr. Ten microliters of the digested product was run on a 3% agarose gel (NuSieve GTG; FMCBI products, Rockland, ME) containing 0.5µg/ml of ethidium bromide and visualized at 312 nm on an ultraviolet trans-illuminator (International Biotechnologies, Inc, New Haven, CT)[14,15,16].

Result

The result of this study indicate that mosquitoes were collected from three sites in Jayapura Municipal. Total a sample 100 were collected. There were 38 materials samples which are positive *Anopheles* from three *Anopheles sp* was identified such as *An. punctulatus* (23), *An. koliensis* (13) and *An. farauti* (2) respectively. See in table 1.

Diversity and dominance of *Anopheles* sp in Jayapura Municipal was found in this study; *An.punctulatus* more higher than *An. koliencis* and *An. farauti*.

Table 1: Species of Anopheles mosquito identified from among those collected in three villages in Jayapura Municipal

Anopheles sp	Located			Frequency	P value
	Hamadi Rawah (%)	Sky Line	Organda		
An.puntulatus	23 (60.5)	0	0	23	0.000
An.koliensis	13 (32.2)	0	0	13	
An.farauti	2 (5.3)	0	0	2	
The others species and male *Anopheles*	0	37	25	62	
Total	38 (100)	37	25	100	

The result shown that the species of *A.punctulatus* as much as 23 (60.5%) were found more higher than *A. koliensis* as much as 13(34.2%) and *A.farauti* 2(5.3%) and others species and male *Anopheles* which exclude of this research as mush as 100 samples.P<0.05.

Based on PCR product shown that genetic diversity from species of *Anopheles* mosquito after giving restriction enzyme ALL1 for cutting of DNA length target band ladder (λ =432bp) for *P. falciparum* and *P. vivax* with band ladder 342bp and108 bp.See in Fig 1 and Fig 2

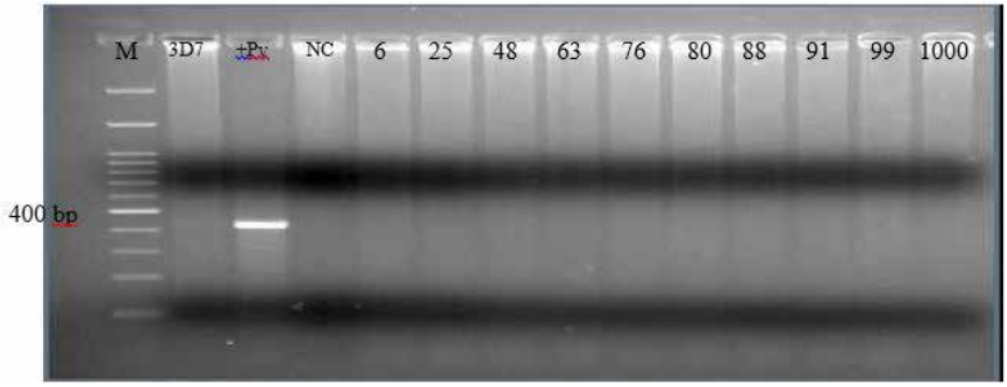

Figure 1: Electrophoregram result show which are PCR product from *Anopheles sp* there were of number 6,48 and 63 are *An.koliensis*, and then number 25,76,80,88,91 and 100 are *An.punctulatus*. M is ladder marker with 100bp, 3D7 was strain of *P. falciparum* (3D7 *strain*) as positive control and *+PV (P. vivax)*, positive control from hospital sample. NC is negative control. Based on PCR result of above was not founded there is DNA band of *P. falciparum* and *P. vivax*.

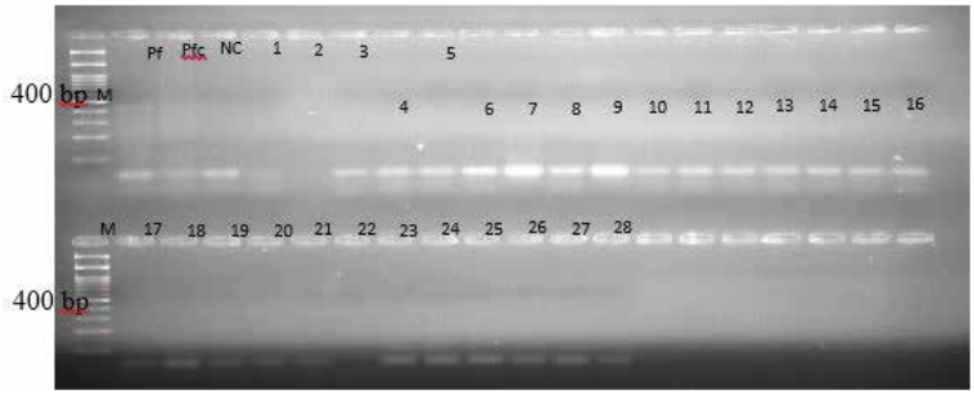

Figure 2: Electrophoregram result show which are PCR produce from species of *Anopheles* mosquitothere were of (number 1,2 are *An. farauti}*, (number 3,4,5,6, 7,8,9.10,11. 12,13,14, 15,16 and 17 are *An. punctulatus*) and (number 18,19,20, 21,22,23, 24,25,26,27, 28 are *An.koliensis*). Pf 1/10 = *P. falciparum*-1 (*Pf/Pfc* is positive control) and *Pfv* 1/10 : mixture between *P. falciparum* and *P. vivax*, negative control (NC). Legend : M: 100 bp ladder marker. Based on PCR product of above was not founded there were DNA band of *P. falciparum* and *P.vivax*

Discussion

Diversity of *Anopheles* sp was found in this study with high dominance in Jayapura Municipal is *An. punctulatus* than *An. koliensis* and *An. farauti*. The high dominance *An. punctulatus* in Hamadi rawah shown that genetic diversity of *An. punctulatus* have the effect of capacity of numbers were indicated the occurrence of transmission and nature of the ability of vector in transmitted malaria[12,13].

The result of DNA Extraction are using a PCR product with direct PCR phusion kit (thermo) and with using the Mito F370 and Mito F5904 primers and there were 38 samples that have been DNA isolated but did not found *Plasmodium* as cause of probably samples without containing *Plasmodium*. It must be fresh or stored frozen to prevent protein degradation; moreover, difficulties in storage arise when working in the field and according[14] species of *An.punctulatus* complex were readily distinguished using a PCR-RFLP analysis based on the ITS2 region of the rDNA. The mosquito samples did not require a particular storage condition because air dried samples contain ample template to generate a PCR product whether the DNA was extracted or a segment of the mosquito was used[14,15,16].

Conclusions

Dominance of *Anopheles* sp in Jayapura Municipal was found in this study; *An.punctulatus* more highly than *An. koliensis* and *An. farauti* because of *An.punctulatus* is a primary of malaria vector and habitat of *An. puntulatus* is in the open pool with clear of pool water or murky the absence of aquatic vegetation, puddles former or human[12,13].

Genetic diversity of *Anopheles* mosquito species based on PCR product of *Anopheles* overall we were not found DNA bands of *P. falciparum and P.vivax* because probably *Anopheles* which such the blood of the captured person has not been infected with *P. falciparum* and *P. vivax*[15,16,17]. The absence of DNA template bands or deletion and insertion at the primer attachment site[14]. DNA insertion can be lead to a change in the size of the DNA fragment, via simple base alterations or band to DNA fragment and the Anopheles sp are not founded more in Jayapura Municipal.

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