Malaria Risk Factors and Mapping in Amfoang Barat Daya-Kupang Nusa Tenggara Timur

Dwita Anastasia Deo[1*], Mahardika Agus Wijayanti[2], Elsa Herdiana Murhandarwati[2]

[1]Faculty of Medicine, Universitas Nusa Cendana. [2]Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada.

Corresponding author: dwita.anastasia@yahoo.com

ABSTRACT

Introduction: Amfoang District is one of malaria-endemic areas in East Nusa Tenggara Province. The areas are adjacent to the shore at the lowland and adjacent to the forest at the highland respectively. Residential areas are surrounded by forest, shrubs, field, streams and pools of water. Most population work as farmers, have low education levels and do not use mosquito bed net

Objectives: This study aims to investigate the factors that influence the incidence of malaria risk and determine the pattern of clustering of cases in the Amfoang Barat Daya, District of Kupang, Nusa Tenggara Timur.

Methods: Mass blood surveys were conducted to investigate malaria cases in three villages in October 2011, January 2012 and April 2012. The 3,515 blood samples were collected for thick blood film. Risk factors were identified through questionares and OR were calculated. Significance was assumed at $p < 0.05$. Coordinates were taken from residence of patients by GPS and analyzed with Purely Spatial Bernaully model to investigate any cluster of malaria cases. River with potentials breeding place was buffered 1km by ArcGis to determine whether malaria cases were in the mosquito fly distance areas.

Results: Dry land farmer, education, malaria symptomatic, habitual of sleep outdoors, farming and fishing (06.00pm-06.00 am), outdoors gathering (06.00pm-06.00 am), wooden wall, mosquito wire, ventilation, plafond, animal shelter distance, breeding place and climate were insignificant for prevalence of malaria. Bed nets and breeding place were significant for prevalence of malaria. Potential of malaria vectors (An. barbirostris and An. sundaicus) were found in lowlands but was not found in highlands. Bed nets is protective factor from incidence of malaria. One primer most likely cluster and 3 secondary clusters were insignificant.

Conclusion : Lowland has risk from malaria. The use of bed nets during 06.00pm – 06.00am is as protective factor for malaria OR 0.1.

Keyword: Amfoang Barat Daya, risk factor malaria, thick blood smear, Anopheles sp., bed nets, mapping

INTISARI

Pendahuluan: Amfoang barat daya adalah salah satu daerah endemis malaria di Kupang, Propinsi Nusa Tenggara Timur. Daerah ini berbatasan dengan pantai pada dataran rendahnya dan dengan hutan pada dataran tingginya. Tempat tinggal penduduk dikelilingi hutan, semak belukar, kebun, aliran sungai maupun sumber mata air. Kebanyakan penduduk bekerja sebagai petani, tingkat pendidikan rendah dan belum menggunakan kelambu anti nyamuk.

Tujuan: Tujuan penelitian ini adalah untuk mengidentifikasi beberapa faktor yang mempengaruhi insidensi malaria dan menetapkan pola kluster kasus malaria yang terjadi di Amfoang Barat Daya, District of Kupang, Nusa Tenggara Timur.

Metode: Mass blood survey dilakukan untuk mengidentifikasi kasus malaria pada tiga desa pada Oktober 2011, Januari 2012 and April 2012. sebanyak The 3.515 sampel darah dikoleksi untuk pembuatan sediaan darah tebal. faktor resiko diidentifikasi dengan quesioner dilanjutkan dengan penghitungan OR. Kebermaknaan ditetapkan apabila $p < 0.05$. Koordinat penderita dipetakan dengan dari tempat tinggal penderita dengan GPS dan dianalisa dengan Purely Spatial Bernaully model untuk mengetahui kluster kasus malaria. Sungai yang berpotensi sebagai tempat perindukan nyamuk dibuffer seluas 1 km dengan ArcGis untuk menentukan apakah kasus malaria yang terjadi terdapat di dalam jarak terbang nyamuk.
Hasil: Petani tegalan, tingkat pendidikan, simptom malaria, kebiasaan tidur di luar rumah, bertani dan pekerjaan sebagai nelayan (06.00 sore-06.00 pagi), kebiasaan berkumpul di luar rumah (06.00 sore-06.00 pagi), rumah terbuat dari dinding kayu, kawat nyamuk, ventilasi, plafon, jarak dengan kandang ternak, dan musim tidak berhubungan secara bermakna pada prevalensi malaria. Kelambu, dan tempat perindukan nyamuk bermakna pada prevalensi malaria. Malaria vektor yang potensial (An. barbirostris and An. sundaicus) ditemukan di dataran rendah tetapi tidak di dataran tinggi. Kelambu merupakan faktor protektive terhadap malaria. Pada penelitian ini diidentifikasi satu kluster primer dan 3 kluster sekunder yang tidak bermakna.
Simpulan: dataran rendah merupakan faktor resiko malaria. Penggunaan kelambu pada jam tertentu (06.00 sore - 06.00 pagi) merupakan faktor protektif terhadap malaria, OR 0.1.

Kata kunci : Amfoang Barat Daya, faktor resiko malaria, sediaan darah tebal, , Anopheles sp.,kelambu, pemetaan

INTRODUCTION

Amfoang Barat Daya district is one of malaria endemic area in Nusa Tenggara Timur province with API (Annual Parasite Index) reach to 58%[0] in 2010, with the population of 3,943 and area of 167,61 km^2. The area is rural area with highland and shore. The area Tis adjacent to the shore at the lowland and adjacent to the forest at the highland and each village is separated by river. Tropic climate with short rainfall. Residents live in group, with the dominant job is as dry land farmers and the side job is as fisherman. Residential areas are surrounded by pools of water in rainy season, farms, bushes and fields. The areas are not far from big cattle (cows, goats and pigs). Walls of the resident house are made by wooden wall (80%), floor land (70%). Residents use clean water for household that comes from rivers and wells. No electricity supply is provided in this area. Most of the residents sleep without bed net and repellent.

Anopheles mosquitoes breeding places and reservoir malaria influence malaria transmission. There are seven species of potential Anopheles as vector in the Timor island of the Province of Nusa Tenggara Timur, namely *An. barbirostris, An. vagus, An. maculatus, An. avularis, An. annularis, An. aconitus* and *An. Subpictus*[3]. *Anopheles barbirostris* has biting character indoor with activity peak at 23.00 p.m-24.00 p.m[4]. The breedings of *An. sundaicus* are in the river banks. Now, although the dry season longer than usual, the cases of malaria remain high. This condition can be caused by asymptomatic patients as a source of infection. In the area with existing seasonal river, puddles could still be formed and become breeding places. as a consequence, malaria remain high although during the dry season[5].

Factors affecting malaria transmission are *Plasmodium* as an agent, mosquitoes and humans as hosts and environments. *Plasmodium* has specific character to each species. This causes different clinical manifestations, such as *P. falciparum* with a short time infection but produces high parasitemia with more severe symptoms. For *P. vivax* and *P. ovale* low parasitemia and symptoms are milder with longer[6] incubation period.

The incidence of malaria in humans associated with innate immunity and adaptive immunity. Innate immunity is found in individual with negative Duffy blood type[7,8]. Hemoglobin S, á, and â thalassemia, G-6-PD deficiency and ovalocytosis resistance to malaria infection[6]. Adaptive immunity has two main features, namely memory and specific. The body makes memory system at first contact with parasite. So, at the moment of contact with the next parasite, the body quickly reacts specifically. The fundamentals character of the active immunity against previous introduction. Passive immunity is given as pre-natal or post-natal from mothers who have high immunity[7,9,10]. Immunity mechanism depends on cellular and humoral factors. Humoral factors associated with antibody and cellular factors associated with lymphocytes, spleen and lymph nodes. Intrinsic factor in human that should also be con**:** sidered are age, gender, race, economic, social, history of previous illness, behaviour and nutritional status.

Malaria is transmitted by several species of female Anophheles mosquitoes through their bite, but malaria can be transmitted directly through blood transfusions or contaminated needles and blood of pregnant women to her baby[7]. Malaria transmission also influenced by geographical conditions, humidity, temperature, rainfall, time, where mosquitoes resting, foraging, breeding grounds. Environmental conditions are conducive for mosquito breeding as well as socio-cultural community. Mosquito's flight range is a very influential factor in the effort of vector to find a place to breeding[10]. Mosquitoes behavior greatly influence the malaria transmission such as living in a house / building (endophilic), outdoors (exophilic), indoors biting (endophagic), outdoors biting (exophagic), biting preference to human (anthropophilic), or both human and animal (zooantropophilic)[11]. Topography type associated with Anopheles species. In West Timor island *An. subpictus* is most common in shore areas, while *An. barbirostris* and *An.vagus* are distributed from the shore to the high plains[4]. Breeding place of *An. Sundaicus* is in lagun[12], but mostly in the estuary of river[5'13]. Freshwater breeding place such as river banks, puddles traces (legs, wheels, pit excavation) are favored by *An. maculatus, An. kochi* and *An. balabacencis* as breeding place[13].

Environmental factors influence the malaria transmission. Temperature greatly affects sporogony cycle. Temperature >30^{0}C and <16^{0}C makes sporogony process is incomplete[6]. Humidity >80% and <60% shortens mosquitoes ages[11]. Rain interspersed with heat affect the development of larvae into adult mosquitoes and heavy rain eliminating the larvae and pupa[14]. High speed of winds reduce mosquitoes activity. Mosquitoes that are in breeding places not tend to leave the place and mosquitoes that are outdoor will tend to enter into the house to escape the wind currents. Low speed of winds cause mosquitoes could fly a long distances and conduct activities to suck blood[15]. Sun light affects the growth of mosquito' larvae in different ways according to its species. There are types that like the shade, while others like open place. Slowly flows of water conditions favored by *An. barbirostris*, while the churning water favored by *An. minimus* and stagnant water conditions favored by *An. letifer*[13] house without a ceiling, ventilation and mosquito' screen increases the risk of malaria transmission[16]. Location of house <5m towards large animal pens can affect the malaria transmission[17]. Natural environment with plants such as mangroves, moss and algae affect the life of the mosquito' larvae because it can block the incoming light. Fish which eat larvae such affect mosquito population in an area. Human behavior shaped by social factors (education, occupation) and cultural factors (habits to stay outdoor the house at dusk / night) is closely related to the incidence of malaria. Education and low income affect the risk of malaria incident[16]. Agricultural work risk to malaria incident. Using repellent, mosquito repellent, and mosquito nets reduce the incidence of malaria[13]. Activity at night, without full body cover affect the risk of malaria incident[19].

This study aims to identify malaria risk factors in Amfoang Barat daya and do health mapping. Health mapping and geographic information system developed by the WHO could help to combat infectious diseases.

MATERIALS AND METHODS

Mass blood surveys were done to two villages (Manubelon and Bioba Baru) in October 2011, January 2012 and April 2012. This is a *cross sectional* study. There are 3,515 blood samples collected, thick bloodsmears were Giemsa stained with 10% Giemsa and examined under a microscope by the author and health officers of Kupang Regency and *cross-checked* by the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada. Positive and negative malaria respondents (age, sex, address had been matched to the positive malaria group) were asked to answer a questionnaire. Independent variable as risk factors were vector, behavior (mosquito repelllent, mosquito netting), house conditions (walls, ventilation, mosquito nets, ceiling), environment (the presence of livestock, puddles, rivers, rice fields, climate) and respondent occupation. The dependent variable incidence of malaria is confirmed positive by microscopic examination. Risk factors is identified through questionnaires and calculated by OR. Significant p value <0.05. Residence coordinate points were taken using GPS, spatially analyzed using *Bernaulli Purely Spatial models* to determine the clustering pattern of malaria cases. Map of the river as a potential larvae breeding places in Buffer 1 km to see cases of malaria in the mosquito flight range.

Adult mosquitoes were collected by aspirator when landing on the arms and legs of catcher. This activity was conducted in lowland and highland at 18:00 to 6:00 in April 2012 and collected every hour. Mosquitoes identification was done using guidelines from Indonesia Health Department[21] in the laboratory of Parasitology Faculty of Medicine, University of Gadjah Mada. Climate data such as temperature, humidity and rainfalls were taken from BMKG of Nusa Tenggara Timur province.

DATA ANALYSIS

Data were analyzed using univariate to obtain a frequency distribution of respondents and the proportion of distribution of positive and negative groups of malaria according to their independent variables that studied. Second phase data is analyzed using bivariate to determine the relationship of the main risk factors associated with the incidence of malaria as well as test the study hypothesis using *Chi-square* test and to interpret the relationship of risk in this study used Odds Ratio (OR). Spatial analysis with *Purely Spatial Bernaulli* model to determine the clustering of malaria cases.

RESULTS AND DISCUSSIONS

Three thousand three hundred and fifteen blood samples were examined with a microscope, 30 blood samples were positive malaria. In October 2011 in the village of Manubelon there 14 (14/1219) blood samples were positive malaria, in the village of Bioba Baru 2 (2/253) blood samples were positive malaria and in the village of Nefuneut no blood samples of positive malaria. In January 2012 in the village of Manubelon, 2 (2/432) blood samples were positive malaria, in the village of Bioba Baru, 2 (2/84) blood samples were positive and in the village of Nefuneut mass blood survey was not conducted due to rainfall in January which resulted in high river overflowed so disconnected transportation. In April 2012 in the village of Manubelon there are 9 (9/978) positive blood samples, in the village of Bioba Baru, 1 (1/221) blood sample was positive and in the village Nefuneut was not found positive sample.

In the dry season of October 2011 there were 16 cases and in the rainy season of January and April 2012 there were 14 cases. The Proportion of malaria in the dry season 0.9% (16/1671), while in the rainy season 0.7% (14/1843). The proportion of malaria cases were the most <16 years ie 53.3% (16/30) and male was the most positive of malaria.

There 8 symtoms were found in positive and negative malaria respondent, presented in Table 1.

Table 1. Symtomatic of positive and negative malaria respondent on Study and Mapping of Risk Factors of Malaria in Amfoang Barat daya-Kupang, NTT in October 2011, January 2012 and April 2012

Symtomatic	Negative Malaria	Positive Malaria	Malaria Falciparum, vivax,mixed (falciparum + vivax)
Fever	125	7	falciparum, vivax
Cephalgia	47	1	mixed (falciparum + vivax)
Myalgia	12	2	falciparum, vivax
Pale	6	1	falciparum
Icteric	2	1	falciparum
Anorecxia	6	3	falciparum
Fatigue	30	3	falciparum
Acites	2	0	-
Splenomegali	1	1(H2)	falciparum
Asymtomatic	3256	23	mixed (falciparum + vivax)

Table 1 shown that there were 8 symtom of malaria respondents. There were 5 symtomatic respondent on falciparum malaria, 2 symtomatic respondent on vivax malaria and 1 mixed (falciparum + vivax) symtomatic respondent. There were 23 asymtomatic respondent of positive malaria.

Parasite density on asymtomatic respondent of falciparum and vivax were higher than on symtomatic respondent. Parasite density on *P. falciparum* higher than *P. vivax* presented in Table 2.

The youngest symtommatic malaria respondent was 3 years old with parasite density (tropozoit of *P. falciparum*) 59.707µl. The highest density parasite (tropozoit of *P. falciparum*) was in asymtomatic respondent that 33 years old, female. In asymptomatic respondents falciparum density was common compare to vivax malaria, means that *P. falciparum* could produce higher parasitemia than *P. vivax.* Mild symtomatic manifestation in positive malaria falciparum in this case might be caused by the respondence imunity.

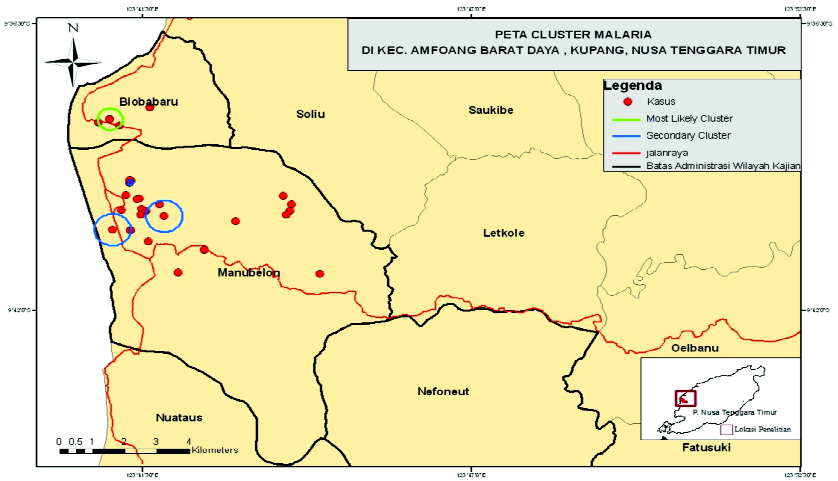

Figure 1. Malaria cluster in Amfoang Barat Daya-Kupang, Nusa Tenggara Timur in October 2011, January 2012 and April 2012

Table 2. Parasite density on Study and Mapping of Risk Factors of Malaria in Barat Daya-Kupang, NTT in October 2011, January 2012 and April 2012.

No	Age (yr)	Sex		Gamet Falciparum (µl)	Trofozoit Falciparum (µl)	Trofozoit Vivax (µl)	Village	Time
		Male	Female					
1	6*	M		120	48760	-	Manubelon	19/10/2011
2	18	M		906	-	-	Manubelon	19/10/2011
3	18	M		14880	1240	-	Manubelon	19/10/2011
4	3*		F	-	59707	-	Manubelon	19/10/2011
5	30	M		-	585	-	Manubelon	19/10/2011
6	8*	M		230	-	4038	Bioba Baru	21/10/2011
7	13		F	356	-	14891	Manubelon	19/10/2011
8	13		F	316	1900	-	Manubelon	19/10/2011
9	30	M		-	1400	-	Manubelon	20/10/2011
10	9*	M		-	8520	-	Manubelon	19/10/2011
11	12	M		-	9120	-	Manubelon	19/10/2011
12	9		F	-	-	3422	Manubelon	19/10/2011
13	48	M		32	-	-	Manubelon	19/10/2011
14	28		F	-	1294	-	Bioba Baru	21/10/2011
15	9*		F	39	234 1	-	Bioba Baru	21/10/2011
16	31*		F	-	-	2470	Manubelon	20/10/2011
17	7		F	-	-	3921	Manubelon	17/01/2012
18	33		F	-	99773	-	Bioba Baru	18/01/2012
19	19	M		-	46641	-	Bioba Baru	18/01/2012
20	47*		F	-	19760	-	Manubelon	19/10/2011
21	9		F	2078	392	-	Manubelon	12/04/2012
22	10		F	-	10268	-	Manubelon	12/04/2012
23	7	M		4696	-	-	Manubelon	12/04/2012
24	16	M		-	358	-	Manubelon	12/04/2012
25	12	M		327	-	-	Manubelon	12/04/2012
26	7		F	-	313	-	Manubelon	14/04/2012
27	40	M		396	79	-	Manubelon	15/04/2012
28	14		F	1023	15507	-	Manubelon	15/04/2012
29	29	M		-	-	19979	Bioba Baru	12/04/2012
30	25	M		-	200	-	Manubelon	12/04/2012

* Symtomatic Malaria

The thick blood films were examined by author and crosscheck was done by Parasitology Laboratory, Faculty of Medicine, Universitas Gadjah Mada. Thirty blood samples were positive malaria parasite. The example of Plasmodium parasite found in thick blood slide are depicted in Figure 2, 3, and 4.

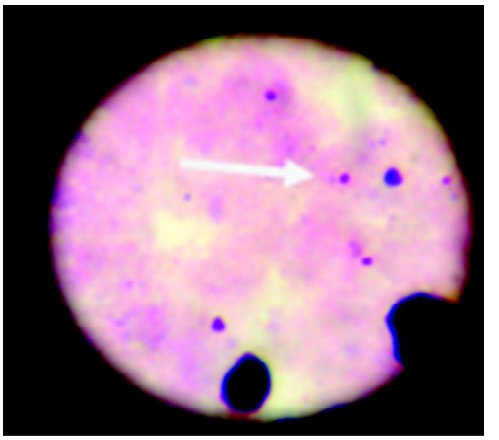

Figure 2. *P. Falciparum* tropozoit, infected erytrocit normal size, thin cytoplasma

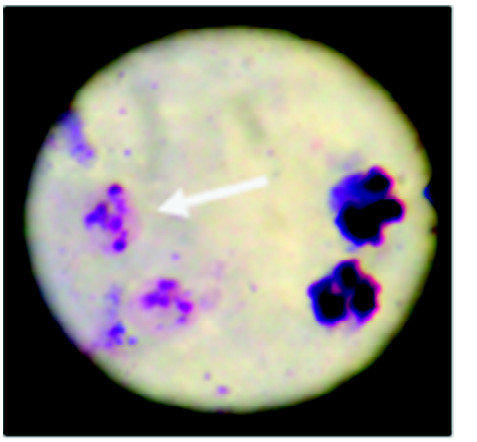

Figure 3. *P.vivax* tropozoit, infected erytrocit bigger, cytoplasma amueboid

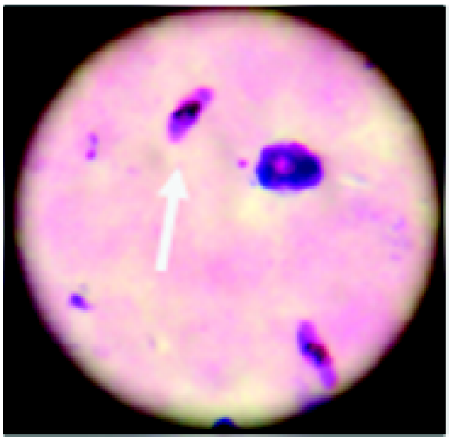

Figure 4. *P. Falciparum* gametocit, erytrocit shape imitate paracite shape, likes sausage

Table 3. Recapitulation of Bivariate analysis on Study and Mapping of Risk Factors of Malaria in Amfoang Barat daya-Kupang, NTT in October 2011, January 2012 and April 2012

Risk Factors	Category	OR	95% CI	P Value	Description
Job	0. risk (dry land farmer)	1,37	0,453 - 4,170	0,573	Insignificant
	1. Not at risk (Civil servant/ student/housewife)				
Net	0. Yes	0,11	0,028 - 0,447	0,001	Significant
	1. No				
Sleep outdoor (18.00 - 06.00)	0. Yes	2,8	0,498 - 15,734	0,228	Insignificant
	1. No				
Mosquito repellent	0. Yes				constant value
	1. No				
Farming and fishing (18.00 - 06.00)	0. Yes	0,48	0,041 - 5,628	0,228	Insignificant
	1. No				
Bath, wash, defecate outside	0. Yes	0,82	0,240 - 2,814	0,754	Insignificant
	1. No				
Assemble / blather out (18.00-06.00)	0. Yes	1,96	0,702 - 5,479	0,196	Insignificant
	1. No				
Walls made of bebak (tree fronds lures)	0. Yes	0,17	0,019 - 1,576	0,085	Insignificant
	1. No				
mosquito net	0. Yes				constant value
	1. No				
Ceiling	0. Yes	0,96	0,905 - 1,033	0,313	Insignificant
	1. No				
Distance existence of large livestock	0. Yes	3,05	0, 969 - 9,657	0,05	Insignificant
	1. No				
Temperature	0. Supported (20°C-30°C)				constant value
	1. Not supported (< 20° atau > 30°C)				
Rainfall	1. Supported (< 60% atau > 80%)				constant value
	1.Not supported (< 150mm)				
Humidity	0.Supported (60% - 80%)	1	0,257 - 3,888	1	Insignificant
	1. Not supported (<60%, >80%)				

Table 3 presented risk factors in bivariate analysis.Only one significant variable that is using nets with p> 0.05, OR 0.11 with 95% CI 0.028 to 0.447.

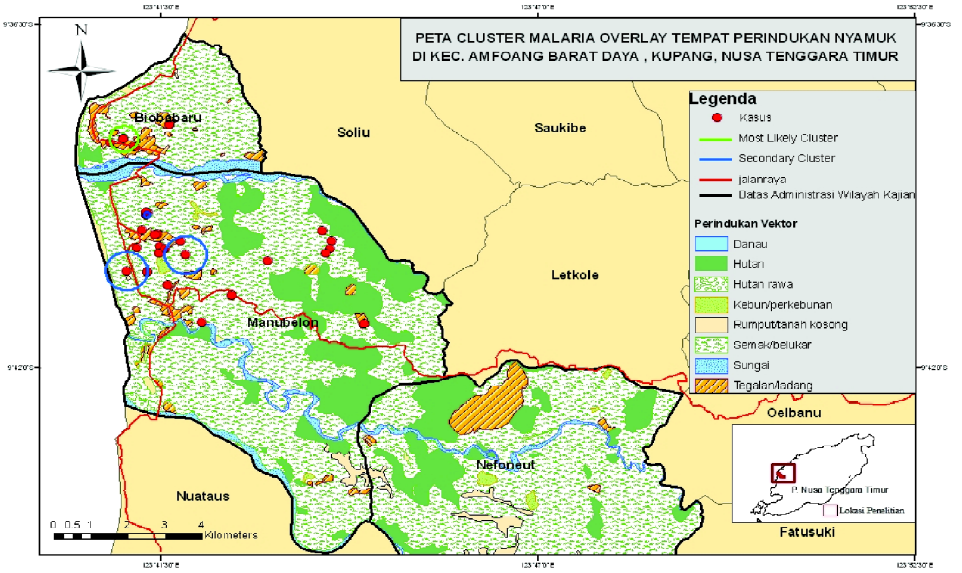

Figure 5. Mapping of malaria cluster overlay with mosquito breeding place Amfoang Barat Daya-Kupang, Nusa Tenggara Timur in October, January 2012 and April 2012

At the end of the study, mosquito species were collected, namely ***An. barbirostris*** and ***An. sundaicus***, indoors at 23.00-24.00 in the lowlands while In the highlands, mosquito species Cx. fatigan, Cx. fuscocefalus were found indoors and and outdoors (Table 4).

Table 4. Types of mosquito species that caught on Study and Mapping Malaria Risk Factors in Amfoang Barat daya-Kupang NTT in April 2012

Time	Manubelon		Nefuneut	
	Indoor	Outdoor	Indoor	Outdoor
18.00-19.00	3 *Cx. fatigan*	3 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fatigan*
19.00-20.00	1 *Cx. fatigan*	8 *Cx. fatigan*	1 *Cx. fuscosefalus* 2 *Cx. fatigan*	1 *Cx. fuscosefalus*
20.00-21.00	6 *Cx. fatigan*	5 *Cx. fatigan*	5 *Cx. fatigan* 1 *Cx. fatigan*	1 *Cx. fatigan*
21.00-22.00	0	6 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fuscosefalus*
22.00-23.00	1 *Cx. fatigan*	3 *Cx. fatigan*	1*Cx .fuscosefalus*	1 *Cx. fatigan*
	6 *Cx. fatigan,,*	10 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fatigan*
23.00-24.00	1 ***An. sundaicus*** 1 ***An. arbirostris***	0	0	1 *Cx. fuscosefalus*
00.00-01.00	2 *Cx. fatigan* 1 *Cx. Trifaeniorchincus*	4 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fatigan* 1 *Cx. fuscosefalus*
01.00-02.00	4 *Cx. fatigan*	5 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fatigan*
02.00 03.00	0	6 *Cx. fatigan*	1 *Cx. fatigan*	1 *Cx. fuscosefalus*
03.00-04.00	0	0	1 *Cx. fatigan*	1 *Cx. fuscosefalus*
05.00-06.00	1 *Cx. fuscosefalus*	0	1 *Ae. vexan*	1 *Cx. fatigan*

An. barbirostris is characterised by its palpi which has no pale strip and proboscis with hard fur: costa with dark spot less than 4, with pale abdomen. *An. sundaicus* was characterised by its palpi has pale strip on apical and subapical, costa with dark spot of 2 and 6, legs with pale spot. Figure 6 and 7 were mosquito species found in Amfoang Barat daya District on April 2012.

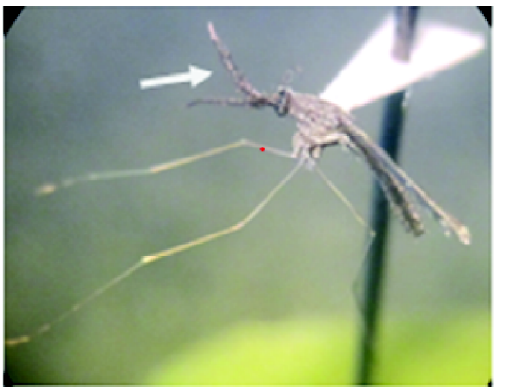

Figure 6. *An. barbirostris*

Figure 7. *An. sundaicus*

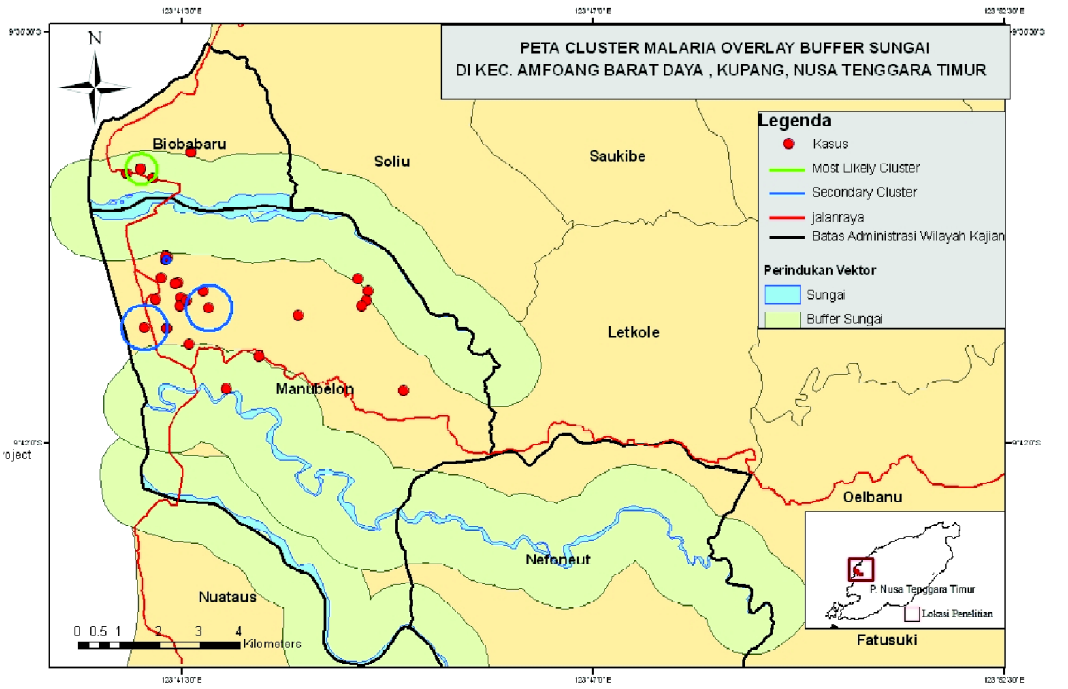

Figure 8. Mapping of malaria cluster overlay with buffered river (1km) Amfoang Barat Daya -Kupang, NTT in October, January 2012 and April 2012

By mapping the river and buffering it for 1 km, it is shown that a positive malaria group residence is within the area of the mosquito flight range with 1-2km radius. The coordinates of residence in spatial analysis using Bernaulli Purely Spatial models, the result is 3 secondary cluster in the village Manubelon with coordinates / radius is (9.674176 S, 123 683 434 E) / 0.56km, p = 0.998; (9.659156 S, 123.688350 E) / 0.079km , p = 0.998 and (9.669850 S, 123 697 779 E) / 0.56km, p = 0.998. Primary cluster in the village of Bioba Baru with coordinates / radius (9.638867 S, 123.682747 E) / 0.38km p = 0.906.

DISCUSSION

Malaria cases in Manubelon village, an area with a population density of 30 per km2, were 1.1% (14/1219) in October 2011 (dry season), 0.4% (2/432) in January 2012 (rainy season) and 0.9% (9/978) in April 2012 (rainy season). Based on the observation, most residents had fields and preferring to stay in the fields when planting season and harvest season. Most adults would rather sleep outside at night when the temperature is hot. Dense settlements were located around the main street with some damaged road conditions. On the rainy season, the damaged roads formed water puddles potential as a mosquito breedin at the end of the study, mosquito species were collected, namely An. barbirostris and An.sundaicus, indoors at 23.00-24.00 in the lowlands while In the highlands, mosquito species Cx. fatigan, Cx. fuscocefalus were found indoors and and outdoors (Table 4) sites.

Most adults did not like to use nets as it was hot/uncomfortable. Anopheles which found potential as vectors that is *An. barbirostris* and *An. sundaicus*. Breeding place of *An. barbirostris* is in the freshwater form fields, and slow current rivers[26]. and *An. sundaicus* like in brackish water, such as mouth breeding river[14]. All were available in rural areas of Manubelon throughout the year.

Malaria cases in Bioba Baru Village, an area with a population density of 33 km2, were 0.5% (2/353) in October 2011 (dry season), 2% (2/84) in January 2012 and 0.4% (1/221) in April 2012 (rainy season). Based on observation, most Bioba Baru population grows rice on the home yard. Netting was only use at the age of <12 years. Residents liked doing fishing at the river at dusk or morning. Denser settlement was located around the mouth of the river. Estuaries is favorable by *An. sundaicus* as breeding place[14].

Nefuneut village (plateau) had a population density of 11 per km2. Since 2000 there has been no malaria patients in the village. In this study there were no malaria patients in the dry season in 2011. Residential area was adjacent to the forest, living in clusters. Residents work as farmers and cattle ranchers. Almost all residents of the village do their activity in the village. They went to the lower land (Manubelon) when buying their needs or going to the clinic in the morning and usually at 15:00 they were back to their village. Based on the observations, most residents slept without net. Cattles and sheeps were more commonly found around the residential area than in Manubelon and Bioba Baru. Potential mosquitoes were more attracted to cattle, buffalo and goats and could be the reasons of no malaria transmission occur.

Children in the District of Amfoang Barat Daya did their activity outdoor including collecting firewood, clean water, feeding animal and doing other social activities. This situation appears possible to get mosquito bites of malaria vector (*An. sundaicus*) which was more outdoor and enter the house at night for blood[12]. In this study, most of positive malaria patient's is males. In the district of Amfoang Barat daya, men had more active outdoor activities until the night than women. The main work of men were gardening, fishing and other social activities, while the women's main job were cooking and taking care of children and weaving. The condition makes men had greater chance of mosquito exposure vector of *An. sundaicus* which is exophagic and endofagic at night[12] and endofagik *An. barbirostris* with peak of biting at 23.00-01.00[3]. Adult men who were sleeping

without mosquito nets would also higher the chance for malaria risk.

Dry land farmers were not significant as risk factors for the incidence of malaria, in contrast to studies in East Kupang in 2001[19]. The difference results of this study could be the topography, vector dynamics and methods used. East Kupang is area of rice fields, when water is always available in the ditch. Transmission of malaria in the East Kupang occurred during the move at dusk until the evening. While malaria transmission occurs in Manubelon and Bioba Baru, mostly were within homes. This study used a *cross sectional* study with primary data while study in East Kupang used *retrospective* and secondary data.

The use of mosquito net at 18:00 to 06:00, according to the results of this study was protective (OR 0.1) on the incidence of malaria. Based on the observation, residents who used bed nets are the children <10 years and adults who have children under five. Based on the observation, the increased of mosquito density was during the rainy season, so many people use the nets during the rainy season than during the dry season.

Habit of sleeping outside between 6:00 p.m. to 6:00 a.m. in this study was not significant as a risk factor for the incidence of malaria. This situation occur due to the residents habit to sleep outdoor when the temperature is hot at night (>30°C), while in this study in October 2011, January 2012 and April 2012 the temperature is below 30°C.

The use of mosquito fuels, farming and fishing at 18:00 to 06:00, this study was not significant as a risk factor for the incidence of malaria. This study is different from study in East Kupang District. This difference due to the sample taken only a part of the population in the District of Amfoang Barat daya.

Bathing behavior, wash and defecate outside the house at 6:00 p.m. to 06:00 a.m, in this study were not significant as malaria risk factors. This study is in line with study in East Kupang District[19]. Behavior of potential malaria vector in the Amfoang Barat Daya District such endophylic, zooantrophylic and peak of biting at night so malaria transmission does not occur.

Respondent housings of *bebak* (tree leaves), did not use mosquito wire for home ventilation, did not use the ceiling were not a risk factor for the incidence of malaria. This study is different from the study in Jaya Pura city. In this study, residence with positive or negative malaria were living in the same location, as a result, housing condition was found to be statistically insignificant with malaria incidence. Breeding places such as rivers, pools, springs and puddle for bivariate statistically was not significant as a risk factor for the incidence of malaria with the similar reasons as above.

Distance of cattle and pigs existence in this study was not significant as a risk factor for the incidence of malaria. This study is different from Wonosobo Regency[18]. *An. barbirostris* in West Timor Island, province of East Nusa Tenggara, have anthrophylic character but according to Depkes[12] and Kemenkes[27] it is zooantrophylic.

The climate, temperature and rainfall did not have a relationship with the incidence of malaria. Malaria positive group and negative malaria have the same situation, so it is statistically has constant value. Humidity does not significantly affect the incidence of malaria. Climate (humidity and rainfall) in this study is the average value of all the islands of East Nusa Tenggara and only one office of Climatology Agency, so the humidity and rainfall data is not accurate to reflect the weather in the Amfoang Barat daya District, while for temperatures taken from BMKG (Meteorology, Geophysic, and Climatology Agency) and measured solely by the author.

Mosquitoe collection was done in April 2012 under strong wind, and raining condition. The collection was conducted once while the house is open only get 2 potential malaria vectors.

Larvae collection with dipper from the pools and rivers in the dry season, while in the rainy season it was found in the river, tire tracks of cars, on the road and in the fields. Larvae collection from the river banks in April 2012 was canceled because it's heavy current flow.

Spatial analysis found one primary cluster in Bioba Baru village and 3 secondary clusters in Manubelon village was not statistically significant (p> 0.05). However, grouping of positive malaria in Manubelon village and Bioba Baru, related to vector breeding places such as fields, lagoon, and river pools might remain vigilant with larger sample sizes.

When it was buffered, 1km from the river, positive malaria cases appear to be in the range of mosquito flight range of 2-3km from the breeding. Bioba Baru and Manubelon Village were close to the estuary of the river. One of the potential vectors of malaria in the district of the Amfoang Barat daya *is An. sundaicus* that use brackish water as their breeding sites. The damaged city's main roads were acted as, potential breeding places of malaria vectors particularly in the rainy season.

CONCLUSION

Lowland adjacent to the beach and close to estuary of the river within 1-2km, at risk of malaria. The incidence of malaria in the village of Manubelon and Bioba Baru was not influenced by the seasons as incidence of malaria occured in both dry and rainy seasons. Existence of seasonal rivers, puddles and pools were favorable as mosquito breeding places. Netting is needed to reduce the incidence of malaria as it was related with the behavior of the malaria vector potential in the Southwest District Amfoang (endophilic).

ACKNOWLEDGEMENTS

We thank Ministry of National Education Directorate through BPPS funding. Nusa Tenggara Timur Province Health Office, Kupang Regency and Manubelon Health Centers. All participants and all the people involved in this study.

REFERENCES

1. Dinkes.Prov. NTT. Laporan kegiatan pemberantasan malaria. Kupang: Dinas Kesehatan Provinsi NTT, 2010.
2. BPS Kab. Kupang 2010. Laporan statistik Kabupaten Kupang tahun 2010. Oelamasi: Badan Statistik Kabupaten Kupang, 2010.
3. Ndoen E, Wild C, Dale P, Sipe N, Dale M. Relation between Anopheline mosquitoes and topography in West Timor and Java, Indonesia. *Malaria Jurnal* 2010;9:242.
4. Ndoen E, Wild C, Dale P, Sipe N, Dale M. Dusk to dawn activity patterns of Anopheline mosquitoes in West Timor and Java, Indonesia. *Southeast Asian J Trop Med Public Health*, 2011;42(3):550-61.
5. Munif A Pranoto. Korelasi musim terhadap populasi tiga vektor malaria-kaitannya dengan insiden malaria di dua kecamatan di Banjarnegara. Provided in discussion Parasitology VIII P4I 1993 Agt 23-25; Denpasar. Available from : URL:http://www.kalbe.co.id acessed on 13 Juli 2012.
6. White J. Malaria. In: Cook GC, Zumla AI, editors.Manson's Tropical Disease. Cina: Saunders Elsevier, 2009: 1201-1300.
7. Bruce-Chwatt. Essential malariology. Oxford: Alden Press, 1985.
8. White NJ, Breman JG. Malaria. In: Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL, Loscalzo J, editors. Harisson's Principle of Internal Medicine. 17th ed. London: McGraw Hill, 2008.
9. Mc Gregor IA. Epidemiology malaria and pregnancy. Am J Trop Med Hyg 1984; 33:517-525.
10. Gill G, Beeching N, editors. Malaria. In: Tropical Medicine. Liverpool : Blacwell, 2009; 9:55-71.
11. Depkes RI. Entomologi malaria. Jakarta : Ditjen PPM & PL, 2003.
12. Depkes RI. Entomologi malaria. Jakarta : Ditjen PPM & PL, 2007.
13. Sari CIN. Pengaruh lingkungan terhadap perkembangan penyakit malaria dan demam berdarah dengue [disertation]. Bogor: Intitut Pertanian Bogor,2005.
14. Harijanto PN. Malaria, epidemiologi, patogenesis, manifestasi klinis dan penanganan. Jakarta : EGC, 2000.
15. Gillies MT. Anopheline mosquitos, vector behavior and bionomic. In: Wernsdorfer WH, McGregor I, editors. Malaria: Principles and Practise of Malariology. Edinburgh : Churchill Livingstone, 1998;453:485.

16. Arsin AA. Analisis pengaruh faktor iklim terhadap kejadian malaria di pulau Kapoposang Kabupaten Pangkajane kepulauan Sulawesi Selatan, Jurnal Kedokteran Yarsi 2006;14(1):46-54.
17. Babba I, Hadisaputro S, Sawansi S. Faktor-faktor risiko yang mempengaruhi kejadian malaria di wilayah kerja puskesmas Hamadi Kota Jayapura [tesis]. Semarang : Universitas Semarang, 2006.
18. Hidayati. Faktor-faktor yang berhubungan dengan kejadian malaria di puskesmas kepil I Kabupaten Wonoobo [tessis]. Semarang: Universitas Diponegoro, 2004.
19. Budarja. Faktor yang mempengaruhi kejadian malaria. Kajian terhadap lingkungan dan perilaku pekerja agraris dan non agraris di Kecamatan Kupang Timur, Kabupaten Kupang, Propinsi NTT [tessis]. Yogyakarta : Universitas Gadjah Mada, 2001.
20. Mirino RRB. Faktor lingkungan rumah dan perilaku masyarakat yang berhubungan dengan kejadian malaria di Kecamatan Agats Kabupaten Asmat, Papua [tessis]. Semarang : Universitas Diponegoro, 2009.
21. O'connor C, Soepanto A. Illustrated Key to Female Anophelines of Indonesia. Jakarta: Direktorat Jenderal PPM&PLP and US Naval Medical Study Unit No 2 Detachment, 1989.
22. Gunawan, S. Epidemiologi malaria. Jakarta: EGC, 2000.
23. Zulkarnaen I, Setiawan B. Malaria berat. In: Sudoyo AW, editor. Ilmu Penyakit Dalam. 4th ed. Jakarta: Departemen Penyakit Dalam, 2006.
24. Natadisastra D, Agoes R, editors. Penyakit oeh sporozoa darah dan jaringan. In: Parastologi Kedokteran Ditinjau dari Organ Tubuh yang Diserang. Jakarta: EGC, 2009.
25. Boundin C, Chumtazi B, Dzigiel M, Peyron F, Picot S, Hogh B, Ambroise-Thomas P. Possible role of specific imunoglobulin M antibodies to Plasmodium falciparum antigens in immunoprotection of humans living in a hyperendemic area, Burkina Faso. J Clin. Microbiol 31: 636-641.
26. Bonne-Webster J, Swellen-Grebel N H. The anopheline mosquitoes of the Indo-Australian region. J H de Bussy 201. Amsterdam, 1953.
27. Kementerian Kesehatan R I. Atlas Vektor Penyakit di Indonesia. Litbangkes RI. Jakarta, 2011