The effectiveness of Insecticide-Treated Nets applications in malaria endemic areas of Tanah Bumbu District

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ABSTRACT

Background : Disease control is transmitted by mosquitoes are mostly done, one of them the use of insecticide-treated bed nets, mosquito nets coated with mosquito repellent and not harmful to health. The aim of this research is to know the effectiveness of application of insecticide treated mosquito nets in malaria vector control in Tanah Bumbu Regency.
Methods :Quantitative research methods, cross sectional design and quasi experiments. Population, the entire population get a mosquito net. Samples, residents willing to take blood, interviewed. Determination of sample behavior and Mass Blood Survey of 100 respondents using purposive sampling.
Results: MBS 100 samples, positive 7 respondents 2 org plasmodium vivax, 5 org plasmodium falciparum. The result of the behavior of insecticide treated bed nets (53%) of respondents received and used bed nets and (47%) received and not used. Result of bio essay test 20 mosquito net and 2 control show 5 mosquito net still effective killing An mosquito. Aconitus, with 82,67- 100% mortality, while 15 other bed nets are ineffective.
Conclusion : The conclusion is the decrease of malaria case after use of mosquito net with SPR 7%. Recommendation, to forest workers to keep wearing insecticide-treated bed nets, using repellents and taking prophylactic drugs while working in the forest.

Keywords: Effectiveness, insecticide-treated nets, Anopheles

Introduction

As one of the source of health problem in Indonesia, malaria still become concern especially for islands outside Java and Bali. Malaria is an acute or chronic infectious disease caused by plasmodium, characterized by several symptoms such as periodic fever, chills, sweating, faintness, anemia and hepatosplenomegaly (Rempengan, 2010). This disease spread through transmission by a particular type of mosquito (Anopheles). Unlike the common mosquito (Culex), this species is particularly bites at night with a typical position where the posterior facing upward at a 48° angle (Tan Hoan, 2007).

Malaria is transmitted to people through the bite of female Anopheles mosquitoes which carries Plasmodium sporozoite. Specific Anopheles that spread malaria are different between one areas to other, there were more than 15 species of Anopheles reported as malaria vector in Indonesia. Malaria transmission can also happen when asexual form of parasite enter human blood through blood transfusion, injection or through the placenta.

Within 2009-2010, number of new cases outside Java-Bali was 45,2‰, nearly 6 times the value of Java-Bali (7,6‰). This condition was due to the presence of malaria vectors and resistance to drugs and insecticides used in vector control. Thus, malaria become one of the infectious diseases that were targeted priority global commitment in the Millennium Development Goals (MDGs). Several attempts were made to control malaria including ITNs for the population with higher risk, insecticide spray (Indoor Residual Spray), larvicides, patient surveillance and the effective treatment with Artemisinin-based Combination Therapy (ACT) as well as preventive treatment in pregnant women. These efforts were accomplished using various sources of funding, both from government and non-government agencies like the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) (Badan Litbangkes, 2010).

One of the protective actions against mosquitoes was by using mosquito net during night, either insecticide-treated or untreated. Mosquito net is a tool that has been used for ages. Applicable net has to fulfil the requirements to have 6-8 holes per centimeter with a diameter between 1,2 to 1,5 mm. There are two types of nets often used by people, one that does not use insecticide and one that impregnated with insecticide. (Lamaka, cited in Izhar, 2010). According to WHO (2007), there are currently two types of impregnated nets: Long-Lasting Insecticidal Nets (LLINs) and Insecticide Treated Nets (ITNs).

The effectiveness of ITNs to reduce malaria prevalence was assumed to be influenced by the behavior of people in using the nets such as installing and washing. This behaviors can differ in each region with accordance to culture and dissimilarity of tribes/ethnic (predisposing factor) (Notoatmodjo, 2003).

Each type (brand) of ITNs may have a different washing instruction. ITNs available on the market were PermaNet® (deltamethrin), Olyset® (permethrin), Interceptor® (α-sipermetrin) and NetProtect® (deltamethrin). Sumitomo Chemical Company (Japan) recommended that Olyset® require heat treatment (heat assisted regeneration) after washing by wrapping the net with plastic bag and left under sunlight to re-enhance the biological activity of its insecticide (Sudarnika et al., 2008). ITNs can last for up to 3-4 years (polyester) and 4-5 years (polyethylene). Improper application and washing might reduce the insecticide contained by ITNs, resulting the ineffectiveness in mosquito control or eventually causing mosquitos resistance against active insecticide compound.

To keep the effectiveness of ITNs in preventing mosquito bites, the application of nets should consider the following issues:

a) Prior to use, newly opened ITNs should be aerated in the shaded place by hanging the nets until the smell is removed (overnight).
b) Mosquito nets are assembled by attaching its four ropes to a bed pole or on a nail in the wall. During use, the lower end of nets is inserted (folded) under the mattress or mat to prevent mosquitos for entering the nets.
c) Mosquito nets should be use in bedtime every night throughout the year, not just during mosquito annoy or deemed no mosquitos.
d) Mosquito nets should be treated with care in order to keep it from torn, hence in the daylight it should be tied up or roll up.
e) In case of the impregnated nets are no longer effective, either LLIN (after 3 years) or ITNs (after 6-12 months), contact a trained officer in Public Health Care (Puskesmas; INA) or local cadres to have a re-dipping.
f) As the nets are flammable, do not smoke or light a fire inside or near the nets (Depkes RI, 2009).

Blum (1974) states that the factor that has the most influence on malaria incidence besides behavioral factor was environmental factor. Environmental factor include conditions that related to the existence of vectors that transmit malaria and how they behave. Therefore, we have conducted a research regarding the effectiveness of ITNs against malaria vector in South Kalimantan.

Research Methods

This research has received ethical approval from the Ethical Committee for Health Research Board No. LB.02.01/5.2/KE.025/2016 on February 1, 2016. This was a quantitative and quasi-experimental research with descriptive approach, using cross-sectional analytic design. Samplings were conducted during March-November 2016 in 3 malaria endemic village of Mentewe Sub-district, Tanah Bumbu District. Samples were 100 respondents who have received ITN distributed by Tanah Bumbu District Health Office, represented as cases and controls. Since the population size was unknown and determined based on the proportion, minimum sample size was

estimated by using the formula of Lemeshow, et al. (1990) and Murti (2006). Samples chosen was defined by criteria: local resident ; aged between 15 to 50 years old, not pregnant or lactating women, and willing to participate in the general requirements that must be met by research subjects for inclusion in the study.

Samples used in this research were purposively chosen. Independent variable was the behavior of the use of ITNs, including acceptance and self-reliance. The dependent variable were the result of the bioassay test netting and the number of malaria cases. Independent variable specified in a quantitative questionnaire. Analysis of the research data presented descriptively in tabular form to determine the proportion of each variable studied and proceed to bivariate analysis to determine the relationship of each independent variable on the dependent variable (95% confidence level).

Tools and materials used in this study were: new ITNs; questionnaires of mosquito nets use in the community; sets of mass blood survey and staining kit (object glass, slides box, vaksinostil/blood lancet, RDTs dipstick, cotton, 70% alcohol, blood sampling form, Giemsa, 10 cc and 100 cc measuring glasses, pipettes, roll tissue, staining box, and distilled water), compound microscope, dissecting microscope, entomology kit (dipper, aspirator, paper cup, identification book, bioassay equipment [cone, laboratory-colonized mosquito, aspirator, timer, sugar, paper cup]), and ELISA analysis kit.

New LLIN were given to the respondents as an exchange for their mosquito nets taken for sampling. Size (length x width x height), type of insecticide-treated nets (brand) and the insecticide active compound became consideration in the replacement. Nets sampling were followed by a check list questionnaire of the respondent's behavior related to the ITNs use. These data include:

a. Number of mosquito nets distributed each family;
b. Duration of mosquito nets usage by the family;
c. Washing frequency over the use of mosquito nets;
d. Washing method and soap used (formulas and trademarks);
e. Application (applied every night or not);
f. Family members who use the nets during sleep;
g. Acceptance and self-reliance of society to the mosquito nets distributed.

Bioassay test was done to measure the effectiveness of insecticide-treated nets (LLIN) after the use by the respondent. It was compliant to the method from WHO (2005) using laboratory-strain of malaria-vector mosquitoes with following procedure:

1. Prepare unfed laboratory-colonized mosquito (An. aconitus);
2. Tested mosquito nets assembled;
3. Plastic cone attached to treatment and control mosquito net surface (3 cones each);

4. Five laboratory-colonized mosquitos were put into each cone (15 mosquitoes per-net) and left exposed for 3 minutes;
5. Mosquitoes from previous were returned in the paper cup and reared for 24 hours in the laboratory. During the test, humidity and temperature were measured;
6. Number of dead mosquitoes was recorded after 24 hours. Residues of insecticides considered to be effective if the mosquito mortality > 70%.
7. In addition, if mortality rate in control mosquitoes were:

   <5%: research can be continued

   5-20%: corrected with Abbot Formula (1)

$$X = \frac{a-b}{100-b} \times 100\% \quad \ldots\ldots\ldots\ldots (1)$$

With:

X = percentage of dead mosquitoes after correction

a = percentage of dead mosquitos at treatment

b = percentage of dead mosquitoes in control

(If mortality in the control mosquito > 20%, the study considered fail and should be repeated).

Behavior of potential malaria-vector mosquitoes was also observed by collecting data on the physical environment (temperature, humidity, rainfall and light intensity) using thermometer, hygrometer, psychrometer and lux meter, mosquito larvae survey, survey of adult mosquitoes indoor and outdoor at night, collection of resting mosquitoes indoor and around the animal cages at night, and morning collection of resting mosquitoes in three purposively-selected houses.

Procedure for ELISA analysis to detect Plasmodium sporozoite on adult mosquitoes were as follows:

1. Collected mosquitoes were put into vial tube and separated by place, time of collection, and species (coded).
2. Head and thorax were separated and ground until smooth.
3. Homogenize 5 ml PBS with:
4. 20 µl Mab capture (for Plasmodium falciparum); or
5. 5 µl Mab capture (for Plasmodium vivax)
6. 50 µl of previous mixture was inserted into microplate well.
7. Cover microplate with aluminum foil and let stand for 30 minutes.
8. Remaining capture in microplate was disposed .
9. 200 µl blocking buffer was added to the microplate well.
10. Cover the plate with aluminum foil and let stand for 1 hour.
11. Discard the rest of the blocking buffer microplate.
12. Add 50 µl mosquito samples into the microplate. Positive control was inserted into well A1, while negative control was inserted into well B1 to H1. Collected samples were inserted into remaining wells.
13. Cover microplate with aluminum foil and let stand for 2 hours.

14. Discard remaining mixture from the plate, wash 3 times using Plate Washer.
15. 50 µl of Mab peroxidase was added into microplate wells.
16. Cover microplate with aluminum foil and let stand for 1 hour.
17. Discard the remaining solution on the microplate, wash 3 times using Plate Washer.
18. Add 100 µl of substrate solution into microplate wells.
19. Cover microplate with aluminum foil, let stand for 30-60 minutes.
20. Results were read using ELISA reader.

Mass Blood Survey (MBS) to determine the number of malaria cases (people whose blood was positive for Plasmodium) were done referring to the guidelines of General Director of P2M and PLP Year 1999. We prepared thick and thin blood smear slides and stained with Giemsa. If the slides were positive, type of Plasmodium and life stage were recorded.

Results

We have collected 90 impregnated-nets from respondents within the working area of Puskesmas Mentewe. Most of the respondents (53%) known to receive and utilize the net, while the rest of respondents (47%) received but did not utilize the net. As much as 20 nets were taken as samples for bioassay analysis. Only 5 nets that were still effective killing laboratory-colonized An. aconitus with mortality rate ranged between 82,67 to 100%. Rapid Diagnostic Test (RDT) and blood slides from 100 samples found 7 positive samples, 5 were positive for P. falciparum and 2 were positive for P. vivax.

Larva survey in breeding sites found 43 and 59 mosquito larvae in first and second survey respectively. We also found other species, Culex as much as 17 and 11 larvae. While in several places we only found II and III instar in the river and drain, IV instar larvae was only found at unused gold mining hole with static water. These places were mostly covered by water plants and food source such as moss and bigger plants as a protection for the mosquito larvae.

ELISA analysis on collected Anopheles mosquito was conducted in the Parasitology Laboratory, Gajah Mada University. Negative result indicated that the Anopheles sp. caught in the field did not act as vectors in Borneo especially Tanah Bumbu District. Detailed ELISA result can be described as follows:

- An. aconitus L18-19 --- Negative for P. falciparum and P. vivax
- An. aconitus L18-19--- Negative for P. falciparum and P. vivax
- An. barbirostris L20-21--- Negative for P. falciparum and P. vivax

Interview using questionnaires for people behavior on ITNs usage was conducted on 100 respondents who did the MBS (RDT and SDJ) consist of 52 men and 48 women,

where most of the respondents (90%) known to have ITNs. Their ITN was taken by our research team as sample and replaced with new ITN with the same brand. All respondents (100%) had heard of malaria, the majority of respondents used ITN (distributed by District Health Office) every night to sleep (59%) , most of the respondents (53%) received and used the ITN, 62% of respondents have washed the ITN, 54% of respondents wash the ITN every less than 6 months, only 41% of the respondents washed the net by soaked with water and detergent, it was then washed by hand and rinsed until the dirt is gone. A small portion (27%) of respondents wash the ITN in the river, and 43% dispose the used rinse water by throwing into the river. 44% of respondents drying the ITN without being exposed to direct sunlight. Additionally, most of the ITNs (52%) were distributed in 2013. Details of people's behavior on ITNs usage can be seen more in Table 1.

Table 1. People's behaviour on Insecticide-Treated Nets in the working area of Mentewe Public Health Center, Mentewe Sub-district, Tanah Bumbu District, Year 2016

No	Characteristic	Option/Answer	Number	(%)
1	Sex	Men	52	52
		Women	48	48
2	Location of sampling	KM 58	34	34
		Bandara dalam Desa Gunung Raya (KM 70)	34	34
		Ata-ata	25	25
		Bandara Luar	7	7
3	ITN taken and replaced	Yes	90	90
		No	10	10
4	History for malaria positive	Yes (cases)	50	50
		No (control)	50	50
5	ITN used every night during sleep	Yes	59	59
		No	41	41
6	Acceptance and independence on distribued ITN	Received and used	53	53
		Received but did not use	47	47
7	ITN washed	Yes		62

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No	Characteristic	Option/Answer	Number	(%)
		No	38	38
8	Frequency of washing the ITN	Once every less than 6 month	54	54
		Once every more than 6 month	30	30
		Never been washed	16	16
9	Washing procedure	Soaked with water and detergent and then handwashed, rinsed until clean	41	41
		Soaked without detergent and then handwashed, rinsed until clean	32	32
		Soaked and rinsed only	16	16
		Dipped in detergent solution (1-2 spoon in 5-10 liter water) directly without pre-soaked until the dirt is removed and rinsed 3 times	11	11
10	Washing location	Bathroom	9	9
		Around the well (place for rinsing)	21	21
		Fish pond	32	32
		River	27	27
		Other place	11	11
11	Place to dispose water after rinsing	River	43	43
		Drain (flow)	26	26
		Pond	11	11
		Pit (static)	8	8
		Digging hole in the yard	9	9
		Other place	3	3
12	Way to dry	Dried under direct sunlight	32	32
		Dried without direct sunlight	44	44
		Dried without hanging	21	21
		Other	3	3
13	Type of malaria	Plasmodium falciparum	5	5
		Plasmodium vivax	2	2
		Negative	93	93

No	Characteristic	Option/Answer	Number	(%)
14	Blood taken	Yes	90	90
		No	10	10
15	Year of ITN distribution	2012	48	48
		2013	52	52

Our interview indicated that there were no particular action to prevent malaria in Mentewe Village and only mosquito coil (burn insecticide) that always be used every night. Only some part of people in Mentewe used ITNs that were distributed by Health Office of Tanah Bumbu District. There was no IRS and prophylaxis drugs for malaria. Statistical analysis showed insignificant relation between ITN usage and malaria cases (p-value = 0,368) where people who slept without ITN were 0,4 times higher risk to be infected by malaria compared to who did (Table 2).

Table 2. Statistical analysis of relation between the use of ITN and malaria incidences

ITN used every night during sleep	Malaria incidences		OR	CI 95%
	Absence	Presence		
Yes	59	3	0,496	0,105<OR<2,343
No	37	4		
p =	0,368			

Statistical analysis showed insignificant relation between acceptance and independence of the respondents on ITNs to the incidence of malaria (p-value = 0,311). People who accepted but did not used the ITN were 2,3 times higher risk to be infected by malaria compare to people who accepted and used the ITN (Table 3).

Table 3. Statistical analysis of relation between acceptance and independence of the respondents and malaria incidences

Acceptance and independence of respondents	Malaria incidence		OR	CI 95%
	Absence	Presence		
Received and used	48	5		
Received but did not used	45	2	2,344	0,433<OR<12,696
p Value =	0,311			

Statistical analysis showed insignificant relation between washing the ITN to malaria incidence (p-value = 0,594). People who washed the ITNs were 1,5 higher risk to malaria compare to people who did not wash the ITN (Table 4).

Table 4. Statistical analysis of relation between activities of washing the ITN to malaria incidence

Washing the ITN	Malaria Incidence		OR	CI 95%
	Absence	Presence		
Yes	57	5	1,579	0,291<OR<8,575
No	36	2		
p Value =	0,594			

Discussion

Most of our respondents worked in the forest as lumberjack, gold miners, seasonal coal miners, and farmers. Research of Suharjo (2010) stated that outdoor activities (work) have high risk to be infected by malaria. Those who stayed overnight at the field, forest, and riverbank have higher chance to mosquitoes bites. Research of Falah and Meiliasari (2013) found that 35,63% respondents who work in the forest and field have high risk for malaria infection (p value : 0,000 OR:7,34 ; 95% CI = 3,55-15,17).

More than half of our respondents (53%) known to received and used ITN that were distributed by Health Office of Tanah Bumbu District. Research of Suharjo (2010) found 77% of 200 respondents in endemic area of malaria in Mentewe Sub-district have used ITNs, while research of Rahmadiliyani and Noralisa (2013) in Teluk Kepayang Village found 208 from 275 respondents have used ITNs.

Bioassay analysis on 20 ITNs showed only 5 nets that still effective killing An. aconitus (mortality rate ranged between 82,67% to 100%), while other 15 nets showed no indication in killing the mosquito. Research of Yahya and Astuti (2010) showed significant relation between the activities of washing the ITNs to mortality rate of laboratory-colonized An. vagus. Research of Barodji and Buwono found that mosquitoes killing activity of PermaNet® "Vestergaard-Frandsen" will decreased after being used for a year either washed or not. It will no longer effective in killing malaria vector An. aconitus (mortality < 70%).

Among 7 positive samples, 5 were positive for P. falciparum, and 2 for P. vivax. Therefore, Parasite Rate in the working area of Mentewe Public Health Center was 7%.

Larvae from both survey locations were colonized at Entomology Laboratory of Balai Litbang P2B2 Tanah Bumbu. Our result found the colonized larvae were An. maculatus, one of malaria vector in Java. There were no study indicating this species to be malaria vector in Kalimantan.

Grasses and bushes were found around the breeding sites. The only larvae predator fish found in some sites were Rasbora sp. Distance between breeding sites to place where the goal miners stay ranged from 5 to 15m. Water pH in most breeding sites were 7 except in the swamp (6,5). Research of Suharjo (2010) indicated that environmental factors of swamps can act as a trigger of malaria. Research of Sitorus (2013) stated that malaria incidence was higher on society with more breeding sites found in their neighborhood. General Director of P&PL (2014) stated that biological environmental factors were effecting malaria incidence due to the presence of mangrove, moss, algae, and many other plants which could help larvae growth by becoming the protection from sunlight or as hideout from predator.

First larvae survey did not found any Culex or Anopheles larvae. This could happen due to the lack of breeding sites on dry season. Moreover,

there was a strong wind and forest fires at that time. Source for malaria spread in Mentewe Village were unused mining hole and land clearing for establishing a camp for the lumberjack. General Director of P&PL (2014) stated that environmental socio-culture factors could affect malaria incidence, these factors might be dam construction, main road construction, mining, and settlement construction for transmigration which could led for environmental changes (Man-made Malaria).

Second larvae survey, mosquito that successfully caught and identified was An. balabacensis. Based on previous study, An. balabacensis was vector for malaria in South Kalimantan especially Tanah Bumbu. Though, ovary and abdomen examination should be done to support this founding.

According to Munif (2009), An. aconitus was malaria vector in highland. This species was exophagic (outdoor biting activity), but will become endophagic if human are their main host. This species was malaria vector in Central, East, and West Java. While An. barbirostris was malaria vector in eastern part of Indonesia and Sulawesi. Malaria vector in Kalimantan were An. balabacensis, An. leucosphyrus, and An. sundaicus, while potential vector were An. letifer and An. tesselatus.

There are two method for mosquito-net distribution: Mass Net Distribution and Integrated Net. Besides mosquito-net distribution, in malaria endemic area such as Mentewe Sub-district, there were also other malaria prevention program which are IRS (Indoor Residual Spraying) twice a year and malaria drugs distribution. However, drug distribution were accommodated only for areas where positive cases found by microscopic analysis. Along with our research, mosquito-net distribution was also done for people in Mentewe who have not received the net before.

Result from Bioassay analysis indicated that only 25% of net samples that were still effective killing An. aconitus. This finding was similar to result from assessment done in Salatiga laboratory where we found that three types of LLIN that have been washed for 5 times were still effective killing An. aconitus. Effectiveness of LLIN (alfa-sipermethrin, deltamethrin, and permethrin) after washed 9 times by Health Cadre, only mosquito-net with deltamethrin (0,055 g/m^2) that was still effective killing An. aconitus (mortality 82,47%).

Conclusions

Result from MBS showed a decrease in malaria cases after the use of LLIN in the society. Olyset mosquito-net was still effective after 4 years usage from 2012, while PermaNet was still effective after 3 years usage from 2013 which consistent to the statement of Malaria Sub-directorate General Director of PP&PL on 2007 that ITNs effectivity will last for 5 years. We recommend to forest workers to keep using ITNs during sleeping at night and use repellent to avoid mosquitoes bite in addition to

prophylaxis consumption prior to work in the forest.

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