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Editor
Praba Ginandjar
Dina Rahayuning Pangestuti
Lintang Dian Saraswati

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Editor
Praba Ginandjar
Dina Rahayuning Pangestuti
Lintang Dian Saraswati

Illustrator
Febri Rijanur

THE ROLE OF *Aedes aegypti* TOWARD DENGUE HEMORRHAGIC FEVER TRANSMISSION IN PURBALINGGA DISTRICT, CENTRAL JAVA PROVINCE

Sunaryo, Bina Ikawati
Animal Borne Disease Control Research and Development Unit, Banjarnegara
Correspondence: yok_ban@yahoo.com

ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is the most a health problem in Indonesia that needs serious attention. DHF in Purbalingga district always high every year, and the cases increased in 2012. This research aimed to know the role of *Ae. aegypti* toward DHF transmission in Purbalingga district.
Method: Survey and observation were done using ovitrap and larvae survey to know entomological parameter. Survey had been done in June 2013 in three endemic DHF villages: Purbalingga Wetan, Kalikabong and Penaruban. DHF cases data obtained from Purbalingga District Health Officer. One ovitrap placed indoor as long as 4 days, larvae survey has been done in 100 houses at every village (total 300 houses).
Results: The result of this research shown that DHF cases were increase in 2013; there were two clusters in Purbalingga and Kalimanah Subdistrict. Cases were predominant by adult, increases in January until May. House Index (HI), Container Index (CI) and Breuteau Index (BI) generally >10 %, which means that the area has risk of DHF transmission. Larvae free index (ABJ) in Purbalingga District were lot (<95 %), there for, community participation is highly expected to eradicate mosquito breeding and increase free number of larvae.
Conclusion: DHF cases in Purbalingga District cluster in two areas ie: Purbalingga and Kalimanah Subdistrict. Dengue case predominant by adult and increases in rain seasson. Entomological parameter generally >10 % including risk area of dengue transmission.
Keyword: *Aedes aegypti*, transmission, Purbalingga

BACKGROUND

Dengue Hemorrhagic Fever (DHF) is the most of public health problem in Indonesia. National incidence rate in 2008 reached 60/100.000 people, with more than 78 % district is transmission area. Three provinces with highest DHF: Jakarta, West Java and Central Java [1]. Incidence rate of DHF in Central Java at last of three years showed: in 2010: 56.8, 2011: 15.3, and 2012: 19.29, while case fatality rate showed in 2010: 1.29 %, 2011: 0.95 %, and 2012: 1.52 %. At many times ago, DHF has spread only in urban areas, but now has spread in all areas. In 2007 from 35 districts in Central Java, 33 districts include as dengue endemic area, and in 2008-2009 has spreads to all districts with highest cases [2].

Purbalingga district one of dengue endemic area in Central Java, since 2009 until 2012, DHF cases always highest: in 2009 recorded 668 cases with 10 dies, in 2010 recorded 488 cases with 8 dies, in 2011 record 108 cases without die and in 2012 record 160 cases with 2 dies [3]. District Health Officer has been done many efforts to stop transmission of dengue i.e. case finding, drug treatment, vector control like

fogging/space spraying, larvacidation and community participation in eradicated mosquito breeding [4].

This research aimed to get information about DHF case situation and find out entomology parameter from ovitrap placement and larvae survey in dengue endemic area. From this research can give information to intervention and control of dengue in Purbalingga district. Important parameter that needs to be knew between the House Index, Container Index, Breuteau Index and Pupae Index. This data can use in the comprehensive intervention of dengue vector control planning.

METHOD

This research was done in June-September 2013 in three villages: Purbalingga Wetan (Purbalingga Subdistrict), Kalikabong (Kalimanah Subdistrict) and Penaruban (Kaligondang Subdistrict). Dengue cases data obtained from District Health Officer (DHO) and Public Health Center in Purbalingga. Environmental spatial data obtained from Bappeda Purbalingga.

Entomological survey (ovitrap placement, total larvae survey) has done in 100 houses in every village (total 300 houses). Identification of container that acted as breeding place was based on type and material. Larvae from container were taken and put in the bottle vial and then to be identified [5,6]. Entomology indicators consisted of: 1. Ovitrap Index (OI): percentage of ovitrap with mosquito's eggs, 2. House Index (HI): percentage of houses infested with larvae and/or pupae, 3. Container Index (CI): percentage of water-holding containers infested with larvae or pupae, 4. Breteau Index (BI): number of positive containers per 100 houses inspected, 5. Pupae Index (PI): number of pupae per 100 houses inspected

RESULTS

Purbalingga District is a 777.65 km2 region. It lays in coordinate 108^{o}11"-109^{o}35" east longitude and 7^{o}10"-7^{o}29" south latitude. Altitude from sea surface between 40-1500 m ASL, almost ¾ of it areas are lowlands. There are two seasons in Purbalingga District: rainy season between January-Juni and dry season between July-December. Rainfall average is 3.739-4.789 mm/year.

Dengue fever was extends distributed in Purbalingga District especially in south area which is urban area with of high density population. There are two cluster DHF case covering Purbalingga Sub district and Kalimanah Sub district (Figure 1).

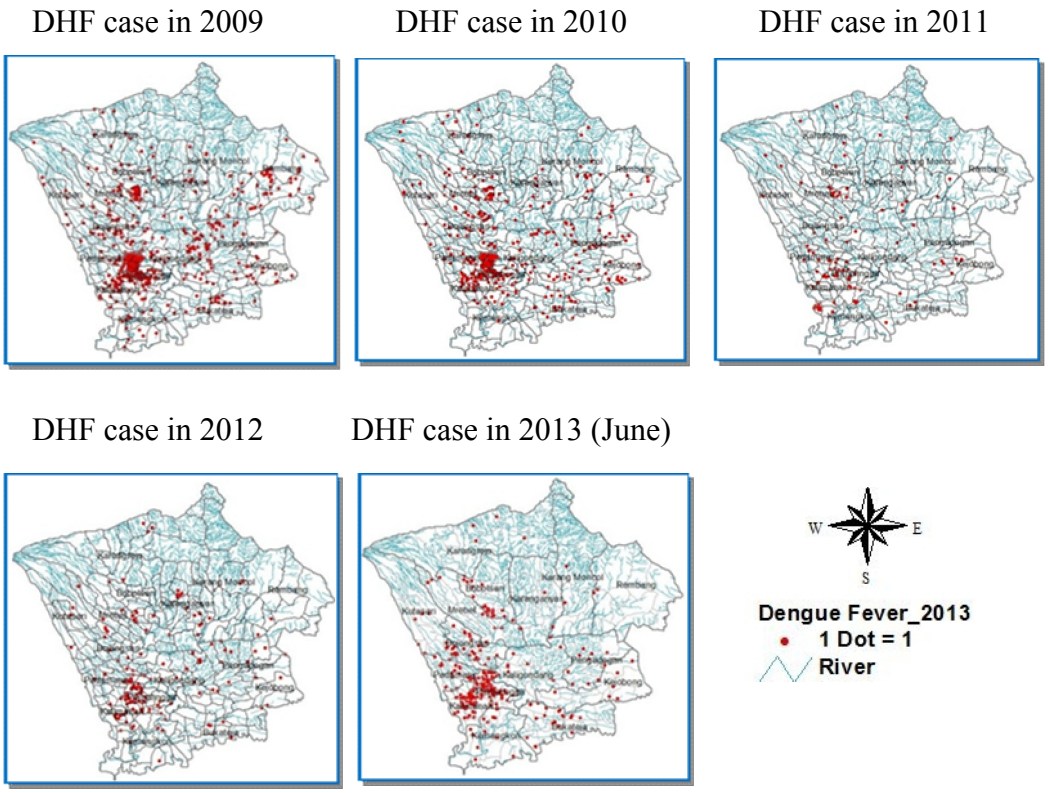

Figure 1. Spatial distribution of DHF case in Purbalingga District (2009-2013 June)

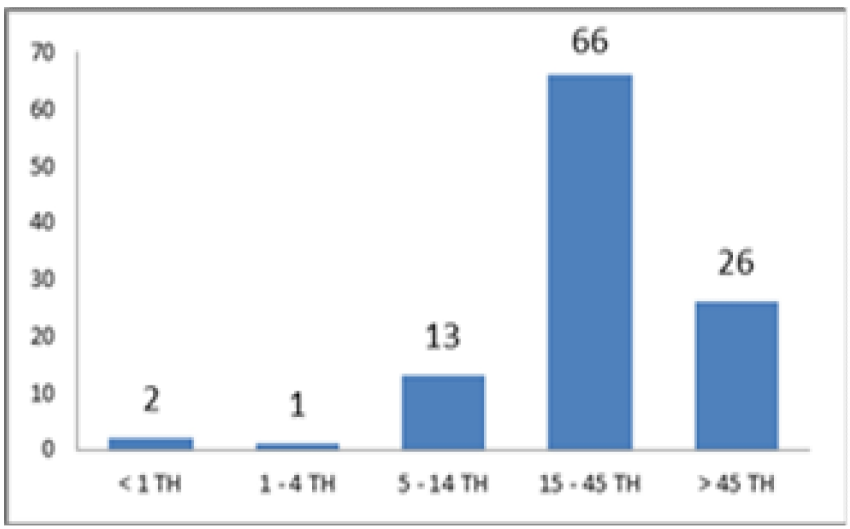

Figure 2. DHF case by age group in Purbalingga district

In general, dengue case in Purbalingga District more suffered at group of adult (age 15-45) compared to other groups. Found case at baby and child is showed in Figure 2. Distribution of DHF case (monthly pattern) in Purbalingga district increased in the early of year between January to May, as the same season pattern in Purbalingga District (Figure 3).

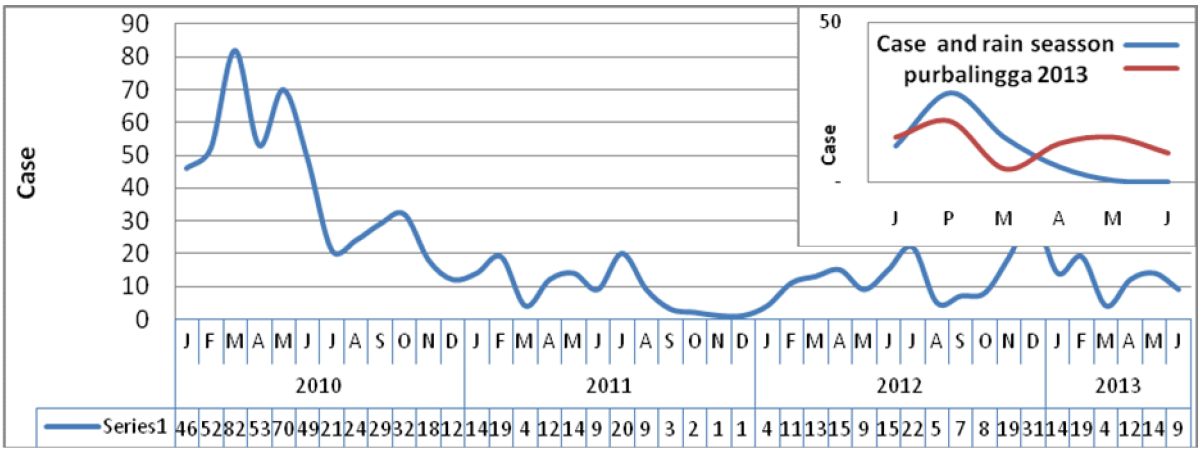

Figure 3. DHF case monthly pattern in Purbalingga district (2010 – 2013)

Entomological survey

Ovitrap placement (300 ovitraps) during 4 days, indoor houses in 3 villages, ovitrap index as: number of positive ovitrap 66 (22%) from 300 ovitraps. Total eggs number of *Ae. aegypti* 1979 eggs. The highest ovitrap number was in Kalikabong Village (40%) and the lowest was in Penaruban Village (6%). The highest number of *Ae. aegypti* eggs came from Kalikabong Village as much as 1190 eggs, while Purbalingga Wetan Village 654 eggs and Penaruban 135 eggs (Table 1).

Table 1. Results of ovitrap placement in 3 villages at Purbalingga district

Village	Ovitrap (+)	OI %	Number of eggs
Purbalingga Wetan	20	20	654
Penaruban	6	6	135
Kalikabong	40	40	1190

Table 2. Indicator of *Ae. aegypti* larvae in 3 villages at Purbalingga district

Village	CI (%)	HI (%)	BI	PI (%)	ABJ (%)
Kalikabong	9.36	18	28	1,67	72
Penaruban	20.92	28	41	6,12	62
Purbalingga Wetan	11.49	17	17	4,05	83

Container Index (CI) = percentage of water-holding containers infested with larvae or pupae; House Index (HI) = percentage of houses infested with larvae and/or pupae; Breteau Index (BI) = number of positive containers per 100 houses inspected; Pupa Index (PI) = number of pupae per 100 houses inspected; Larvae Free Index (ABJ) = percentage of houses infested with larvae/pupae

Index larva from survey in 3 villages describe in Container Index (CI), House Index (HI), Breteau Index (BI), Pupae Index as: The highest container index found in Penaruban village 20.92 %, and the lowest in Kalikabong village 9.36 %. Positive house larvae at highest in Penaruban village 28 %, while in Kalikabong and Purbalingga Wetan each approximately equal 18 % and 17 %. Number of positive containers in every highest house in Penaruban 41/100 houses in Kalikabong 28/100 houses and

Purbalingga Wetan 17/100 house. Number of houses with highest positive pupae was also in Penaruban village 6.12 %, while the lowest was in Kalikabong 1.67 %.

Type of container with positive larvae/pupae of Ae. aegypti

From 643 containers investigated in three villages, 13.37% (86 container) was found to have larvae. Type of controllable site (CS) containers found indoor and outdoor included: WC tub, bathing tub, bucket, dispenser, refrigerator, and flowerpot. While disposable site (DS) container included: used tires, places of toy, coconut shells, ex-drinking water, water waste, used cans, broken jar. Container with most larvae found was bathing tub (30.23%) and bucket (25.58%). Positive container indoor wasmore prominent (59.30%) compared to outdoor. Generally container larvae were positive at light room (87.21%).

DISCUSSION

Dengue case distribution in Purbalingga district from year to year was extension area. There were two clusters i.e.: Purbalingga and Kalimanah Sub districts. Dengue case was predominant in adult. Dengue pattern increased in January until May, but declined in the end of the year. This pattern as the same season, dengue increased of synergistic with rainfall [7].

Potential transmitted of dengue with House Index (HI) parameter

This research revealed that Purbalingga District is a high risk area to dengue transmission, with HI of 17-28% (HI threshold: >10%) [8]. According to WHO, House Index (HI) is an indicator at most applied to monitor infested of mosquito [9,10]. House index is percentage of house which are positive for vector breeding, so can express number of populations at risk [11].

Potential transmitted of dengue with Container Index (CI) parameter

Container index serves the purpose of equipment of important comparator in evaluating of vector control, however fairly not useful from epidemiology view. CI showed the number of larvae positive containers is compared to number of all containers because only showed percentage of larvae positive container [9].

Based on research which has been done by using CI parameter in 3 village in Purbalingga District, in Kalikabong Village showed low risk for dengue transmission, because CI 9,36% (threshold: CI > 10%) [8]. While in Penaruban Village (20.92%) and in Purbalingga Wetan (11.49 %) CI in both the villages showed high risk for dengue transmission.

Potential transmitted of dengue with Breteau Index (BI) parameter

Breteau Index (BI) shows relation between positive containers with number of houses, this index assumed best index, but does not express number of larvae in container. Generally, BI is better indicator than CI and HI because combining between houses and container. Therefore, BI has significant epidemiology value [11].BI in 3 villages in Purbalingga district ranged 17 to 28. Based on WHO criteria, Purbalingga was considered as high risk area. According to WHO, HI > 5% and/or BI > 20 for any locality is an indicator for sensitive and vulnerable to dengue transmission [9]. Density

of pupae in 3 villages location pertained lowest especially in Purbalingga Wetan Village. Existence of pupae indicates that belated container control more than 1 week. Larvae free index (ABJ) at in 3 village pertained has risk to dengue transmitted, because ABJ < 95 %

CONCLUSION

There were two main cluster of DHF case in Purbalingga District i.e: Purbalingga and Kalimanah Sub district. DHF cases predominant in adult and increases in January until May. HI, CI and BI generally >10% (specified by WHO), including risk area dengue transmission. *Ae. aegypti* breeding places are most found in container in door and pertained controllable site that is bathing tub and bucket, which is the cement and plastic.

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REFERENCES

1. Ahmad, S., Suseno, U., Hasnawati, Sugito, Purwanto, H., Brahim, R., Sunaryadi, Sibuea, F.,Pangribowo, S. & Sarijono, Indonesia Public Health Profile, Jakarta, 2009. (Indonesian)
2. Central Java Province Health Officer, Situation of Animal Borne Disease in Central Java Province. 2012. (Indonesian)
3. Purbalingga District Health Officer, Dengue Report in Purbalingga, Purbalingga DHO, 2012. (Indonesian).
4. Ministry of Health Republic of Indonesia. Preventive and Dengue Control in Indonesia, Jakarta: Directorate General of Disease Control and Environmental Health, 2005. (Indonesian)
5. Directorate General of Diseases Control and Environmental Health. Training Modul for Program Handled of Dengue Control in Indonesia. Jakarta. Ministry of Health Republic of Indonesia, 2007. (Indonesian)
6. Directorate General of Diseases Control and Environmental Health. Manual of Entomology Dengue Hemorrhagic Fever survey. Jakarta: Ministry of Health Republic of Indonesia, 2001
7. Nova, Primary Vector of Dengue Identification and Distribution of Dengue Virus in Banjarnegara District, Animal Borne Disease Control Research and Development Unit of Banjarnegara. 2012. (Indonesian)
8. Soedarmo**.** Dengue Haemorrhagic Fever in Childs. Jakarta: University of Indonesia Press, 2005. (Indonesian)
9. WHO**.** Preventive and Control of Dengue Haemorrhagic Fever. Jakarta : WHO and Ministry of Health Republic of Indonesia, 2003. (Indonesian)

10. WHO**.** Prevention and Control of Dengue and Dengue Haemorrhagic Fever. s.l.: WHO Reg, 2000. Pub. SEARO No.29.
11. Ma'mun, Kharis**.** Entomology Survey of Dengue Haemorrhagic Fever and Maya Index in Kalangan village, Baturetno Sub district, Bantul District.Yogyakarta: Faculty of Medicine, Gadjah Mada University, 2007. (Indonesian)
12. Sang G. Purnama and Tri Baskoro, Maya Index and Density of Aedes aegypti larvae to Dengue Infection., Denpasar: Makara Kesehatan, Vol. 16. 2012
13. Pant C.P. and Self L.S**.**Vector Ecology and Bionomic, Monograph on Dengue/Dengue Haemorrhagic Fever. New Delhi: s.n., 1993.

MOSQUITO'S BITE PREVENTION AND BREEDING PLACES ELIMINATION MAY PROTECT FROM DENGUE HEMORRHAGIC FEVER IN SEMARANG CITY 2012: A MULTIVARIATE ANALYSIS

Lintang Dian Saraswati, Praba Ginandjar
Faculty of Public Health, Diponegoro University, Semarang
Correspondence: lintang@undip.ac.id

ABSTRACT

Background: Semarang is one of endemic city with high morbidity and mortality. The incidence cumulative from 2009-2011 were 192.78; 363.75; 84.37 per 100,000 population respectively.
Method A case-control study was conducted to explore the risk factor of dengue infection in Semarang City from January-December 2012. 230 cases and 230 controls were included. Cases were residents of Semarang suffering from DHF in all hospitals, and reported to the DHO of Semarang from January 1, 2012 until December 30, 2012. Controls were neighbors of cases approximately 100 meters and not experiencing DHF symptoms at least 1 month prior to the interview which using questionnaire. Cases obtained from S.0 reported by the hospital to DHO Semarang.
Results: After further adjusting the confounder, there was strong correlation between eliminating mosquito's breeding places (*PSN/Pemberantasan Sarang Nyamuk*) and the use of personal protective equipment (e.g.; repellent, mosquito coil and insecticide hand sprayer) with dengue cases. A person with single PSN estimated to be 2.22 (95% CI:1.32-3.72) times higher risk to have DHF than person who did three PSN. The risk increase to those who did not do PSN (OR 5.85, 95% CI: 2.86 -11.99). A person who used one mosquito's bite prevention estimated to be 4.21 (95% CI: 2.31-7.65) times higher to have DHF compare to those who used three kind of prevention.
Conclusion: The risk of person for DHF is determined by person behavior in eliminating mosquito's breeding places and use of mosquito's bite prevention in Semarang City.
Keywords: dengue, *Aedes aegypti,* breeding places elimination, bite prevention

BACKGROUND

Dengue Hemorrhagic Fever (DHF) is a common disease that of ten occurs in the tropics, and frequently appears in the rainy season. The disease is transmitted through mosquito bites that are *Aedes (Ae. aegypti* and *Ae. albopictus)*. WHO stated that approximately 3 billion people have a risk infected with *dengue virus* and every year there are 50-100 people in the world already infected [1].

Similar conditions also occur in Indonesia, an increase of patients occurred from year to year. In 2011 incidence rate was 27.67/100,000 per population and CFR was 0.91% then in 2012 incidence rate was 37.37/100,000 and CFR was 0.90% [2]. Incidence rate of DHF in Central Java at last of three years showed: in 2010: 56.8, 2011: 15.3 and 2012: 19.29. Case fatality rate in 2010 (1.29%), 2011 (0.95%) and 2012 (1.52%) [3]. Semarang is one of DHF endemic city with high morbidity and mortality.

The incidence cumulative from 2009-2011 were 192.78; 363.75; 84.37 per 100,000 population respectively [4]. There has a tie to eradicate mosquito breeding habits (*Pemberantasan Sarang Nyamuk*/*PNS*) and habits to prevent mosquito bites with the incidence of dengue in the Semarang city in 2012.

The objective of this study was to know the relationship mosquito's bite prevention and breeding places elimination with the incidence of dengue in Semarang city in 2012 after controlled by variable covariates (age, sex, occupation, the presence of water reservoir and a neighbor who had a history of DHF).

METHOD

A case-control study was conducted to explore the risk factor of dengue infection in Semarang City from January-December 2012. 230 cases and 230 controls were included. Cases were residents of Semarang suffering from DHF in all hospitals, and reported to the DHO of Semarang from January 1, 2012 until December 30, 2012. Controls were neighbors of cases approximately 100 meters and not experiencing DHF symptoms at least 1 month prior to the interview which using questionnaire. Cases obtained from S.0 reported by the hospital to DHO Semarang. Multivariate analyzes were performed to obtain the parsimony model of mosquito's bite prevention and breeding places elimination on the incidence of DHF after controlled by covariates variable.

After all candidate variables entered in the model then a multivariate analysis were performed with double logistic regression with methods of backward elimination. Further assessment of were interaction and the assessment results did not reveal any known interactions of variables. The next step was assessing the possibility of confounding. The assessment results that confounding variables on the relationship of mosquito's bite prevention and breeding places elimination with the incidence of dengue in Semarang city in 2012 were neighbors who had a history of DHF illness, presence of objects that can be water reservoir around homes and habits to prevent DHF

RESULT

From table 1 we obtained a model without interaction and we know that there were confounding variables (age, neighbors with DHF, the presence of water reservoir and breeding place elimination) on the relationship of mosquito's bites prevention with the incidence of DHF in Semarang. A final model showed in Table 2 and 3. Table. 2 showed a person with single PSN estimated to be 2.22 (95% CI:1.32-3.72) times higher risk to have DHF than person who did three PSN. The risk increase to those who did not do PSN (OR 5.85, 95% CI: 2.86 -11.99). Table 3 showed a person who used one mosquito's bite prevention estimated to be 4.21 (95% CI: 2.31-7.65) times higher to have DHF compare to those who used three kind of prevention.

Table 1. OR Crude, 95% CI, and p value of relationship between independent variables with DHF incidence before controlled by covariate variables in Semarang 2012

Variables	OR (Crude)	95% CI	p value
Main variables			
Breeding places elimination			
Do 2 of 3	1.00	*Reference*	
Do only 1	2.35	1.45-3.80	0.01
Not doing at all	9.51	4.80-19.56	0.01
Mosquito's bite prevention			
Do 2 of 3	1.00	*Reference*	
Do only 1	4.81	2.82-8.22	0.01
Not doing at all	6.04	3.81-9.60	0.01
Covariates			
Sex			
Female	1.00	*Reference*	
Male	2.22	1.46-3.40	0.01
Age			
≥ 45 year	1.00	*Reference*	
15 – 44 year	0.32	0.20-0.50	0.01
0 – 14 year	4.78	2.92-7.93	0.01
Occupation			
Not at risk of DHF	1	*Reference*	
Risk of DHF	2.03	1.25-3.29	0.01
Neighbors with DHF			
No	1.00	*Reference*	
Yes	3.19	2.06-4.97	0.01
The presence of water reservoir			
No	1.00	*Reference*	
Yes	2.79	1.80-4.31	0.01

*All covariates that have a p value<0.25 will be a candidate as a variable in the model or in modifier effects/interactions

Tabel. 2. A logistic regression models of breeding places elimination with the incidence of dengue in Semarang 2012 after controlled by variable covariates

No	Variables in a model	Coef.	Std. Err.	P>|z|	OR	[95% CI]
1.	**Do breeding place elimination**					
	Do 2 of 3				**1**	*Reference*
	Do only 1	0.80	0.26	0.01	**2.22**	1.32-3.72
	Not doing at all	1.77	0.37	0.01	**5.85**	2.86-11.99
2.	**Neighbors with DHF**	1.02	0.25	0.01	**2.78**	1.69-4.56
3.	**The presence of water reservoir**	0.92	0.25	0.01	**2.50**	1.52-4.12
4.	**Mosquito's bite prevention**					
	Do only 1	1.33	0.28	0.01	**3.79**	2.18-6.58
	Not doing at all	1.89	0.30	0.01	**6.65**	3.67-12.04
	_cons	2.23	0.27			

Log likelihood = -208,97879

Table 3**.** A logistic regression models with mosquito's bite prevention with the incidence of dengue hemorrhagic fever in Semarang in 2012

Variables	Coef	Std.Err.	OR	[95% CI]			P>|z|
Mosquito's bite prevention							
Do 2 of 3			**1**	*Reference*			
Do only 1	1.44	0.30	**4.21**	2.31	-	7.65	0.01
Not doing at all	2.06	0.33	**7.82**	4.12	-	14.86	0.01
Ages							
15–45 year	1.09	0.77	2.98	0.66	-	13.49	0.16
0-14 year	2.78	0.80	16.09	3.38	-	76.56	0.01
Neighbors with DHF	1.05	0.27	2.87	1.70	-	4.85	0.01
The presence of water reservoir	0.85	0.27	2.34	1.37	-	4.01	0.01
Do breeding place elimination							
Do only 1	0.69	0.28	2.00	1.15	-	3.48	0.01
Not doing at all	1.76	0.39	5.80	2.70	-	12.44	0.01
_cons	3.81134	0.80					

Log likelihood = -187,84933

DISCUSSION

The result of breeding place elimination similar with research conducted by Kasdi [5] and Usman [6]. Bad behavior in eradicating larvae provides the risk of developing DBD 8.13 times [5]. Gubler stated prevention and control of dengue vector control depends on *Ae.aegypti* located around the house, where most of the transmission happened [7]. While Halstead stated dengue transmission depends on water saving habits that become breeding places for *Ae. aegypti* which located around the house [8]. Low participation in breeding places elimination occurs because people do not understand and realize the importance of doing it [9], so they do not care with the recommendation by government in conducting breeding places elimination [10]. Benthem *e.tal*. stated person with ignorance of breeding places elimination had 2 times higher risk dengue hemorrhagic fever compared with individuals who care [11].

Mosquito's bite prevention result similar with Yu Chao [12], which stated that individual stake precautions against mosquito bites (use antimosquito, spraying insecticides, using repellent and sleep in mosquito net) will be protected 2 times compared to individuals who do not any perform mosquito bite prevention. Published data [13-17] indicate that repellent efficacy and duration of protection vary considerably among products and among mosquito species. Product efficacy and duration of protection are also markedly affected by ambient temperature, level of activity, amount of perspiration, exposure to water, abrasive removal, and other factors. In general, higher concentrations of active ingredient provide longer duration of protection, regardless of the active ingredient. Products with <10% active ingredient may offer only limited protection, often 1–2 hours. Products that offer sustained-release or controlled-release (microencapsulated) formulations, even with lower active ingredient concentrations, may provide longer protection times.

The important and effective way to prevent DHF in endemic areas is to avoid mosquito bites by using insect repellent and other prevention. According to WHO [14-17], community activities to avoid and kill adult mosquitoes consisting of anti-mosquito chemical burn, to kill or repel mosquitoes, put gauze on house ventilation, especially in the bedroom ventilation, use mosquito spray (hand aerosols) to kill

mosquitoes, use a bed net to protect infants and children from mosquito bites during the day. All of these had been proven in several studies [18-27].

Use of anti-mosquito skin (repellent) is one way that can be done to prevent mosquito bites. Repellent especially useful in the situation of the individual conducting activities such a sat school or work [28]. The suggestion for using repellent i.e. a minimum of 2 (two) times a day in accordance with the peak biting activity of dengue vector that is 2 (two) hours after sunrise and 2 (two) hours before sunset [29]. The use of mosquito repellent fuel/electric will be effective for a person who does sedentary activities such as indoors watching television, studying, etc [30-31]. While the use of anti-mosquito spray insecticide made to kill adult mosquitoes are effectively carried out if there is a known sector in adult mosquitoes [30-31].

CONCLUSION

The risk of person for DHF is determined by person behavior in eliminating mosquito's breeding places and use of mosquito's bite prevention in Semarang City.

REFERENCES

1. World Health Organization. Global strategy for dengue prevention and control 2012-2020. Geneva: WHO, 2012.
2. Andi Muhaidir, presented in Dengue Vaccine Meeting, Brazil, 9-11 April 2013
3. Central Java Province Health Office. Profile of Central Java 2011. Semarang
4. DHO Semarang, 2012. *Dengue cases report in Semarang 2011.*District Health Office Semarang.
5. Kasdi, Muhamad, 2003. *Analisis Faktor Risiko terhadap Kejadian Demam Berdarah DengueDBD di Kota Bontang Tahun 2003*. Available from: http://www.litbang.depkes.go.id/ maskes/052004/ demamberdarahl.htm.
6. Usman, Sarif, 2002, *Faktor Risiko Yang Berhubungan Dengan Kejadian Demam Berdarah Dengue di Kota Semarang Tahun 2002,* Tesis FKM-UI
7. Gubler, Duane J.,1998, *Dengue and Dengue Hemorrhagic Fever*, Clinical Microbiology Reviews, July 1998, p. 480-496, Vol. 11, No. 3
8. Halstead, SB, 1984, *Selected primary healthcare: strategies for control of disease in the developing world.* XI. Dengue. Rev Infect Dis;16:251-64.
9. Lloyd LS. *Best practices for dengue prevention and control in the Americas*. Washington, DC, Environmental Health Project/United States Agency for International Development (USAID), 2003:71.
10. McMichael AJ et al. *Climate change and human health: risks and responses*. Geneva, World Health Organization, 2003.
11. Van Benthem BHB, 2002, *Knowledge and use of prevention measures related to dengue in northern Thailand.Trop Med Int Health.* 2002;7:993–1000. doi: 10.1046/j.1365-3156.2002.00950.x.
12. Chao, Day-Yu, et al, 1998, *Predisposing Factors of Dengue Cases by Random Effect Model in the Largest Dengue Hemorrhagic Fever Epidemic in Taiwan in 1998* , *Dengue Bulletin – Vol 24, 2000*
13. AP Dash, Rajesh Bhatia and NL Kalra. Dengue in South-East Asia: an appraisal of case management and vector control. Dengue Bulletin – 2013: 36: 9-11

14. World Health Organization, Regional Office for South-East Asia. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition. New Delhi: WHO-SEARO, 2011. SEARO Technical Publication Series No. 60.
15. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control. Geneva: WHO/TDR, 2009. Document No. WHO/HTM/NTD/DEN/2009.
16. World Health Organization. Report of the eighth meeting of the global collaboration for development of pesticides for public health, 20-21 February. 2012. Geneva: WHO, 2012.
17. World Health Organization. Integrated vector management. Working Group Meeting Reports. Geneva: WHO, 2009. Document No. WHO/HTM/NTD/VEM/2009.2
18. Acharya A, Goswami K, Srinath S, Goswami A. Awareness about dengue syndrome and related preventive practices amongst residents of an urban resettlement colony of south Delhi. J Vect Borne Dis 2005; 42: 122-7.
19. Brug J, Aro AR, Richardus JH. Risk perceptions and behaviour: towards pandemic control of emerging infectious diseases: international research on risk perception in the control of emerging infectious diseases. Int J Behav Med. 2009; 16(1): 3-6.
20. Itrat A, Khan A, Javaid S, Mahwash K, Khan H, Javed S et al. Knowledge, awareness and practices regarding dengue fever among the adult population of dengue hit cosmopolitan. Plos One. 2008; 3(7): 1-6.
21. Koenraadt C, Tuiten W, Sithiprasasna R, Kijchalao U, Jones J, Scott T. Dengue knowledge and practices and their impact on Aedes aegypti populations in Kamphaeng Phet, Thailand. Am J of Trop Med and Hyg. 2006; 74: 692-700.
22. Pérez-Guerra CL, Zielinski-Gutierrez E, Vargas-Torres D, Clark GG. Community beliefs and practices about dengue in Puerto Rico. Rev Panam Salud Publica. 2009; 25(3): 218-26.
23. Pham HV, Doan HT, Phan TT, Minh NN. Ecological factors associated with dengue fever in a Central Highlands province, Vietnam. BMC Infectious Diseases Journal. 2011; 11(172): 1-6.
24. Phuanukoonnon S, Brough M, Bryan JH. Folk knowledge about dengue mosquitoes and contributions of health belief model in dengue control promotion in Northeast Thailand. Acta Trop. 2006; 99: 6-14.
25. Scott TW, Morrison AC: Longitudinal field studies will guide a paradigm shift in dengue prevention. In: Vector-borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections. Washington, DC: The National Academies Press; 2008. pp.132-149
26. Shuaib F, Todd D, Campbell-Stannett D, Ehiri J, Jolly PE. Knowledge, attitudes and practices regarding dengue infection in Westmoreland, Jamaica. West Indian Med J. 2010; 59(2): 139-46.
27. Tomayao A. People's knowledge and practice and Aedes aegypti infestation in Cebu City, Philippines and implications for community-based dengue control. The Philippine Scientist. 2000; 37: 79-93.
28. Wan Rozita WM, Yap BW, Veronica S, Muhammad AF, Lim KH, Sumami MG. Knowledge, attitude and practice (KAP) survey on dengue fever in an urban Malay

residential area in Kuala Lumpur. Malaysian. J Public Health Medicine. 2006; 6(2): 62-7
29. Van Benthem BH, Khantikul N, Panart K, Kessels PJ, Somboon P, Oskam L. Knowledge and use of prevention measures related to dengue in northern Thailand. Trop Med Int Health. 2002; 7(11): 993-1000.
30. Tsuzuki A, Hunyh T, Tsunoda T, Luu L, Kawada H, Takagi M. Effect of existing practices on reducing Aedes aegypti pre-adults in key breeding containers in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg.2009; 80(5): 752-757.
31. Tun-Lin W, Lenhart A, Nam VS, Rebollar-Téllez E, Morrison AC, Barbazan P, Cote M, Midega J, Sanchez F, Manrique-Saide P, Kroeger A, Nathan MB, Meheus F, Petzold M. Reducing costs and operational constraints of dengue vector control by targeting productive breeding places: a multi-country non-inferiority cluster randomized trial. Trop Med Int Health. 2009; 14(9): 1143-53.

ENVIRONMENTAL VARIABILITY AND HABITAT SUITABILITY OF MALARIA VECTOR IN PURWOREJO DISTRICT, CENTRAL JAVA INDONESIA

Mursid R[1], Sudibyakto H A[2], Gunawan T[2], Sutomo AH[3]
[1]Doctoral Environmental Program Gadjah Mada University, Yogyakarta; Faculty of Public Health, Diponegoro University, Semarang
[2]Faculty of Geography, Gadjah Mada University
[3]Faculty of Medicine, Gadjah Mada University
Correspondence: mursid_rahardjo@yahoo.com

ABSTRACT

Background: Purworejo's landscape was composed the coastal and hilly regions, endemic malaria diseases, influences of global climate change. Characteristic of environmental determines a species Anopheles distribution in space and time. Understanding the ecological habitat of mosquito disease vectors can, therefore, be a powerful control or of the risk of exposure to the pathogens they transmit. However, research of the geographic distribution and ecological requirements of these species is to date still inadequate.
Methods: Breeding and resting quality, indoor-resting mosquitoes were sampled from 34 villages covering of ecological settings available in Purworejo, Central Java. Using a water and air quality parameter, species density on presence records, habitat suitability maps were constructed for habitat suitability index (HSI). Environmental variables as key to mosquito geographic distribution were assessed by cluster and discriminate techniques.
Results: Nine *Anopheline* species were collected, of which 2 are known as vector to transmit malaria in Purworejo. Discriminant factor for *Anopheles* dynamic were Total Dissolve solid (0,737), Chloride (0,943), turbidity (0,733), salinity (0,949), hardness (0,755), conductivity (0,523), dissolved oxygen (0,867), pH (0,796), air temperature (0,837), humidity (0,578), wind speed (0,799). The suitable region of mosquito life, base on Habitat suitability index (HSI) pre-dry season 41%, dry season 65%, pre-rainy season 79%, rainy season 79%
Conclusions: The distribution of major *Anopheles* species in Purworejo is strongly affected by the environmental characteristic dynamic. Habitat Suitability Index (HSI) should help improve malaria vector control interventions by targeting places and time
Keywords: vector malaria, habitat suitability index, Purworejo

BACKGROUND

Climate change impacts various aspects of life [1]. Based on report of the United National Development Project (UNDP) 2007, Indonesia is a country that influenced by climate change [2]. IPCC report (2007) shows the influence of global climate change on the biological and social systems. Extreme weather increases the risk of spread of infectious diseases including diarrhea, vector-based diseases (vector-borne diseases), including malaria, non-communicable diseases, and floods [3]. Several studies have

tested the relationship between variations in weather and the occurrence of infectious diseases. Changes in weather due to El-Nino (ENSO) affects the spread of disease vectors or non-vector-based like malaria, dengue fever, cholera, hantavirus [4,5].

The process of transmission of disease (including malaria), involves three components: the host (host), agent (parasite) and the environment [6-8]. WHO in the "Manual On Practical Entomology in Malaria", adding 2 components in the transmission process, compose it 5 components: 1.parasit; 2.vektor; 3. host; 4. physical environment; 5. factor ot her biological [9]. The environmental factors can be projected in space and time, respectively, periodic and continuous. Therefore transmission of malaria can be predicted periodically [10].

In 2011 the Central Java province experience and outbreak, where the number of Annual Parasite Incidence (API) in several districts has increased. Purworejo is the only region that experienced outbreaks during the period 2009-2011. Three districts namely Loano, Bagelen and Bener have been designated as areas experiencing extraordinary events. At least 311 residents were in the care of hospitalization due to malaria cases [11].

Macro mapping results indicate spread of malaria in coastal areas, surrounding farm land, the region in the hills Menoreh belt. Temporal fluctuations in malaria cases occur in addition Purworejo from year to year, it also happens from month to month. Environmental change and the vulnerability of the population is strongly suspected as a contributing factor of the condition. Fluctuations in malaria cases occur due to an accumulation of various factors that cause the interaction between sp (vector), parasites, and human environment changes from time to time. Increased incidence of malaria was considered related to climate change, as well as environmental changes, such as changes in land use, changes in behavior, and socio-economic changes [12].

Institute of Medicine (IOM) in 2003 conveyed the Convergence model, linking the various causal factors of disease transmission, which were: 1. man; 2. microbe; 3. ecology factors; 4. descent and biological factors; 5. physical environment factors; 6. socio-political and economic factors. Convergence models incorporate various factors by concentric (centered) to support the emergence of malaria cases [13].

Anopheles sp. found in Purworejo recorded more than 5 species [14]. The 2012 study found species 9 species, namely: *An. balabacensis*; *An. aconitus*; *An. maculatus*; *An. vagus*; *An. kochi*; *An. barbirostris*; *An. subpictus*; *An. indifinitus*; *An. annularis* [15]. Each species has its own characteristics, habitats, distribution patterns and bionomic [16]. Bionomic vector of malaria is influenced by various environmental factors. There are significant changes in weather to bionomic malaria vectors [17].

METHOD

Location and sampling

The study was conducted in Purworejo, which is geographically located at 3°23’20”-4°9’35” east longitude and 5°43’30”-6°47’44” south latitude. Purworedjo district consists of 494 villages, has a specific landscape, coast and mountains in the zone of ecosystem. Malaria endemic areas in Purworejo include 233 villages with a height of 0-723 mdpa. Last ten years (2000-2012) there were 171 villages have been cases of malaria, 22 villages of Low Case Incidence (LCI), 18 village as Middle Case Incidence (MCI), and 22 village as High Case Incidence (HCI) [11].

The research of environment quality, type and density of vectors was done in 34 villages in the region Purworejo. Sample grouped and defined by height class interval region with 100 mdpal and endemicity areas with proportional random sampling. Five villages in the sample to represent the region have been no cases of malaria at an altitude of 0-100 mdpal. Region with MCI category, HCL, LCI at altitude 0-100 mdpal represented each 1 sample. Areas with no cases of malaria category at an altitude of 100-200 and 200-300 mdpal, each represented by two samples, while the other categories, namely category regions with LCI, MCI, HCI height for each sample is represented by 1 sample (Figure 1).

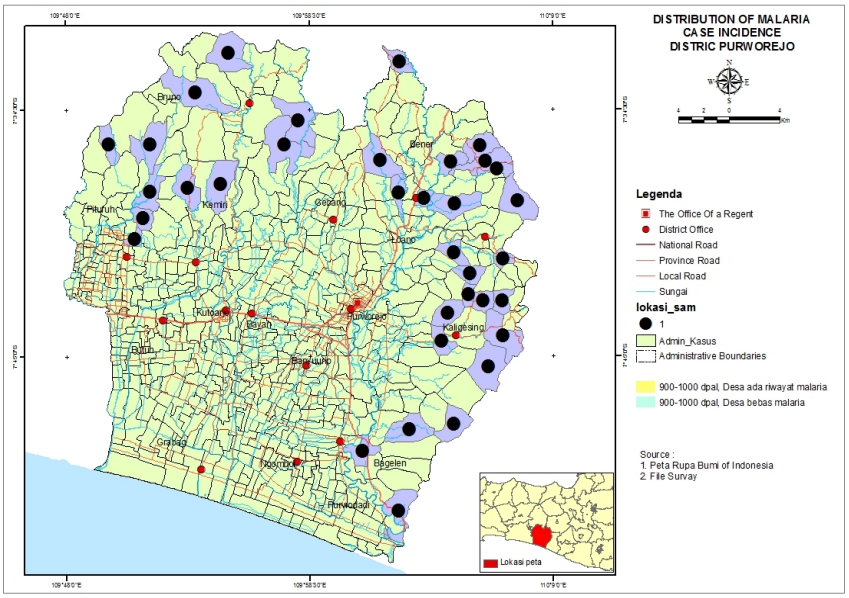

Figure 1 Sampling location

Vector data and environmental quality

The study was conducted during the fourth period:transition of rainy-dry season (May), dry season (July), transition of dry-rainy season (October), and rainy season (December). Type and density of malaria vectors was the parameter in this study. The other parameter were culture, water quality, air quality, breeding places. Vector density was observed by the method of MHD and MBR, whereas the identification of vectors in parasitological laboratory. Observation of the type and density of vectors was done annualy 06:00 pm to 06:00 am. In every village, 2 houses were selected. Each house consisted of 2 catchers, for indoor and outdoor. Confirmatory test was done with ELISA.

Water quality analysis consisted of 9 parameters: pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), Dissolved Oxygen(DO), chloride, electrical conductivity (EC), salinity and hardness. Air quality parameters on resting place consisted of air temperature, humidity and wind speed. The hourly temperature variation by placing a thermometer in each sample village along with vector density measurements .

Environmental Variability analysis results

Environmental variability analysis is done with graphical trend analysis of the dynamics of environmental quality. Range of water quality parameters and the air density is used as a vector for analysis to determine the effect of neighborhood characteristics and density of the vector.

Environmental variabilityand malaria vectors bionomic

Analysis to determine the effect of changes in the environmental characteristics of the malaria vector abundance was conducted by cluster analysis. Cluster and discriminant analysis to determine which parameters influence significanly. Cluster analysis was performed to determine the distribution of classes of water and air quality. Analysis used cross-tabulation (cross tab), and chart dendrogram. Results of both approaches were the similarity of data that can be formed into 5 groups, 4 groups or 3 groups. Members of the group have been shown in the table cluster analysis and dendogram. Discriminant was used to determine the factors that have a dominant influence. The analysis method was a hierarchical method, because the number of clusters has not been determined. It was assumed that these variables contribute to forming the group in vector abundance. Test of equity analysis of group means, resulting Wilks lambda to a variable rate. Using the F test significance>0.05 would result in a significant discriminant. This matrix structure will provide information which variables had a significant role as a discriminant deskriminan and magnitude of each variable.

RESULT

Species diversity and distribution of Anopheles

Observation in transition of dry to rainy season (May) found 9 species of *Anopheles* at 27 locations. The species were as follows: *An. balabacensis*; *An. aconitus; An. barbirostris; An. vagus; An. anularis; An. kochi; An. maculatus; An. indifinitus; An. subpictus*. The most dominant species was *An. vagus* (209 sp), followed by *An. barbirostris* (84 sp) and *An. aconitus* (49 sp). *An. vagus* was found on a wide range of areas, including 17 villages, with a height range < 100 mdpal up to > 700 mdpal. Other species that hadwide distribution was *An. aconitus* and *An. barbirostris*, scattered in 11 (eleven) villages observations. *An. aconitus* found at an altitude of 200-300 and 400-600 mdpal. At a height of < 200 and > 700 mdpal the species was not found. *An. barbirostris* species are found in areas with height range < 100-600 mdpal, while more than 700 mdpal height of the species was not found. Other species found in low populations was *An. indifinitus*. They found in Somoleter and Cepadak (300-500 mdpal). *An. subpictus* species were found at low densities in areas with an altitude of 100-400 mdpal. The highest number of species was found in Wonosido, a location with a height of 400-500 mdpal. At Wonosido we found six species of the *An. aconitus; An. barbirostris; An. vagus; An. anularis; An. kochi; An. maculatus*. However, *Anopheles* was not found in 7 rural areas: Dadirejo (< 100 mdpal); Kapiteran (< 100 mdpal); Ngandagan (< 100 mdpal); Sokoagung (100-200 mdpal); Tepansari (100-200 mdpal); Durensari (300-400 mdpal); Puspo (300-400 mdpal).

Observation in dry season (July) found 8 Anopheles species at 30 locations. Anopheles were not found in four areas: Dadirejo, Bagelen, Kedunggubah and

Hardimulyo. Eight species found were as follows: *An. balabacensis; An. aconitus; An. barbirostris; An. vagus; An. anularis; An. kochi; An. maculatus;* and *An. subpictus*. *An. indifinitus* did not match any arrests in the dry season. The most dominat species caught was *An. barbirostris* (362 sp), followed by *An. aconitus* (216 sp) and *An. vagus*. Spatial distribution of spesies, *An. aconitus* found in a wide range of areas, included 20 villages, with a height range < 100 mdpal up to > 700 mdpal. Other species that widely distributed was *An. barbirostris*, *An. vagus* and *An. maculatus*. *An. barbirostris* found at an altitude of 100-600 mdpal. *An. vagus* species was found in area with a height range < 100-> 700 mdpal, together with *An. maculatus*. Species found in low populations were *An. subpictus* (Ngandagan with a height < 100 mdpal); *An. anularis* (Wonosido, 500-600 mdpal). We also found four areas with the highest number of species: Wonosido (500-600 mdpal), Kaliharjo (100-200 mdpal), Bleber (200-300 mdpal), Kaliglagah (mdpal 200-30). Five species were found in the four areas.

Observation in transition of dry to rainy season (October) found 8 species at 26 locations. The eight species found were as follows: *An. balabacensis; An. aconitus; An. barbirostris; An. vagus; An. anularis; An. kochi; An. maculatus;* and *An. indifinitus*. *An. subpictus* did not match any arrests in the transition of dry-rainy season (October). The most dominant species was *An. vagus* (353 sp), followed by *An. aconitus* (63 sp) and *An. barbirostris* (53 sp). The range of distribution area of *Anopheles vagus* < 100 mdpal up to > 700 mdpal, inlcuded 16 villages. Other species that had wide distribution is *An. aconitus* and *An. barbirostris*. *An.indifinitus* found at less area, this species found at altitude of 300-400 mdpal (Puspo), although, on previous observations of this species was not found. *An. anularis* was found of 100-200 mdpal (Tepansari). *An. kochi* found only at altitudes of 500-600 mdpal (Wonosido, Watuduwur, and Ngadirejo).

Observation on the rainy season (December) found 7 species at 30 locations. We did not find Anopheles species in four rural areas: Kedung Pom Kulon, Pekacangan, Durensari and Puspo. The seven species found were as follows: *An. balabacensis; An. aconitus; An. barbirostris; An. vagus; An. anularis;* and *An. kochi*. *An. subpictus* and *An. indifinitus* did not match any arrests in this season. Dominan species were *An. vagus* (196 sp); *An. aconitus* (77 sp) and *An. balabaensis* (60 sp). Distribution of habitat of *An. vagus* was found on a wide range areas, included 15 villages, with a height range < 100 mdpal up to > 700 mdpal. Other species that have broad distribution is *An. aconitus* (14 observation villages), *An. balabacensis* (13 villages). *An. indifinitus* and *An. maculatus* were not found in the observations of the rainy season. Other species that were found in low populations consisted of *An. anularis* (Kaliglagah, 200-300 mdpal) and Cacaban Lor (300-400 mdpal). Other studies found the area with the highest number of species Kaliglagah observation locations (200-300 mdpal), found as many as 5 species. The species distribution of four survey periode as the Table 1.

Table 1. Malaria species distribution base on elevation of 4 survey periode

No	Lokasi/Desa	Ketinggian	Jumlah Spesies Anopheles																																			
			balabaensis				aconitus				barbirostres				vagus				anularis				kochi				maculatus				indifinitus				subpictus			
		mdpal	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	Bagelen	<100		0											1		3	13																				
2	Dadirejo	<100	0	0	0													1																				
3	Kaliwader	<100			2			1	4	2	14	25			11	12	1	1					3	4					6									
4	Kapiteran	<100	0							1		5	1				9	9										1										
5	Ngandangan	<100	0		0			2				2						2																			1	
6	Bleber	< 100	1					65	3	11	1	85	1	3		2	4							3		1		7	1	1								
7	Kaliharjo	<100		2	0	9		1			27	80			1									2				2										
8	Kaliurip	<100					1		1			1		1	1																							
9	Kaligondang	100-200					5	10	5						21	1							1				5	10	1	1					2			
10	Somoleter	100-200		3	1			1	3	4	1	2	5	2	7		11	3					1						3	26	1				3			
11	Kedunggubah	100-200	1	0	3	2																																
12	Sokoagung	100-200	0			3		8									10	12										3										
13	Tepansari	100-200	0					39	6	21				7		8	79	43			2								4	3								
14	Medono	200-300				1		13		4					18	3	40	27					1															
15	Kaliglagah	200-300	2	2	1	14		45		4		15			6	5	94	52			1							4	2	3								
16	Kedung Pom kulon	200-300		1		0							1		1		9												1									
17	Pekacangan	200-300				0			1						6													3										
18	Sendangsari	200-300			0		2			1	1	1			1	1																						
19	Cacaban Lor	300-400		1	1			2	3		13	1			4	11	10	3			1								2									
20	Durensari	300-400	0	5		0			4																													
21	Cepedak	300-400				1		2	2	4	1	18					1							1							1							
22	Puspo	300-400	0			0		1	1																								1					
23	Ngaran	400-500	3				5	1			1						1								1		1		1	5								
24	Guntur	400-500			0	1	1	3		1						5												1										
25	Hardi mulyo	400-500		0		9	2								5		1	1									1		1									
26	Wonosido	400-500					21	7	3	2	14	35	1	1	113	7	55	17	18	14			15	10	1	1	4		7									
27	Ngadirejo	500-600	1				2	2	1	3	9	12	4	6	10		18	11					2		1	3		1										
28	Ngasinan	500-600			1	1			1	3					1		1							1			1											
29	Purbowono	500-600	1	1	0	5	4																															
30	Watuduwur	500-600					5	1	13	16	2	80	40	12			2								2	1		10										
31	Benowo	600-700	2			1	1		12							1	3	1										2									
32	Donorejo	600-700	1		3	8											1										1										
33	Gunungwangi	600-700		4	0			8							2													1		1							
34	Tlogoguwo	>700			0	5		4																			1										
	Jumlah Spesies	1781	11	14	9	55	49	166	52	55	84	355	52	25	209	48	255	129	18	14	0	2	23	21	4	7	13	30	29	45	2	0	0	0	5	0		

Note : A : observation on May ; B: obseravtion on July; C : observatiom on October
D : observation on December

Analysis ELISA

ELISA test is used to determine the content/levels in the body sporozoites sp. This test refers to ELISA sporozoites detection, as a reference in the test. The test showed Anopheles species acted as transmitting Plasmodium (malaria vectors). Species found during the arrest in rainy-dry transition period (May), dry season (July), dry-rainy transtition (October) and rainy season (December) were as follows: 1. *An. balabacensis*; 2. *An. barbirostris*; 3. *An. vagus*; 4. *An. maculatus*; 5. *An. aconitus*; 6. *An. kochi*; 7. *An. indifinitus*; 8. *An. anularis*; 9. *An. supictus*. ELISA test results showed as table 2.

Table 2. Spesies Anopheles as vector malaria

No	Observation location	Periode	Malaria vector	plasmodium
1	Kedunggubah	October	An. balabaensis	P.falciparum
2	Ngaran	October	An.maculatus	P.falciparum
3	Kaliharjo	December	An.balabaensis	P.vivac
4	Kedunggubah	Descember	An. balabaensis	P.falciparum

Environmental Quality Data
Water quality(breeding place)

1. The degree of acidity (pH)

Results of water quality measurements at 34 locations showed in transition from dry to rainy season (May), pH ranged 4.3 to 8, with an average of 6.63. In the dry season (July) pH ranged 6-8.5, with an average of 7.12. In the dry season pH ranged 4.8 to 8.5 with an average of 6.84. In the rainy season pH ranged 4.3 to 8 with an average of 6.63.The data also showed the rainy season the average degree of acidity (pH) equal to 6.63 lower than 7.12 in dry season and the rainy to dry transition of 6.84. The average pH of rainy with rainy-dry transition was 6.63. This suggested that pH during the rainy to dry transition and dry season were unchanged. pH increased in dry season and rainy season before entering transition. Figure 2 showed the amount of variation in the average pH of 0.49 is the average difference between the rainy and dry seasons.

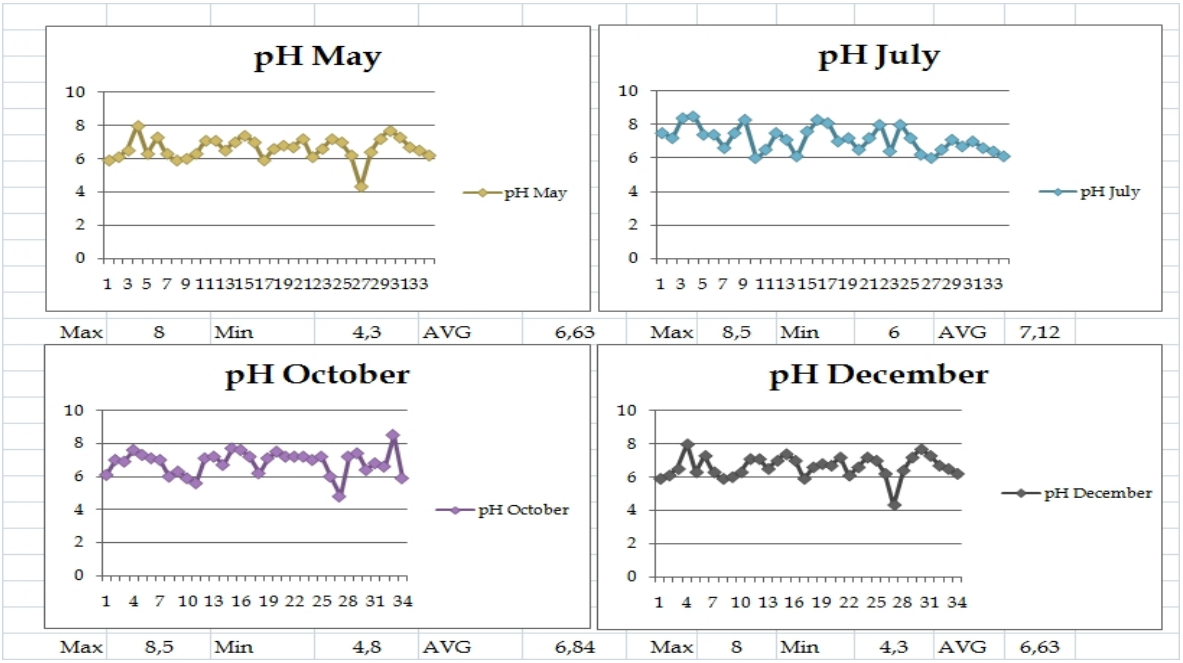

Figure 2. Variability of pH of 4 survey periode

2. Turbidity (NTU)

Our result showed turbidity variation between seasons. The highest mean turbidity (10.3) was found in transition of dry to rainy season (May),while the lowest (4.8) was found in dry season (July).

3. Total dissolved solid (TDS)

In general, a chemical compound derived from the surrounding environment. Our result showed variability of dissolved material (TDS) between seasons. The mean TDS was as follow: 123.4mg/l (May), 154.5mg/l (July), 147.8 mg/l (October) and 102.7 mg/l (December).

4. Suspended solid (TSS)

Measurement in wet-dry transition season (May) showed average TSS was 26.44 mg/l, ranged of 1-87 mg/lt. At follow-up measurements in the dry season (July) the average TSS values decreased to 3.76 mg/l, ranged of 1-31 mg/lt. Deviation between the transition and dry season of 22.68 mg/lt. TSS measurements on dry to rainy transition season (October) produces an average value of 13.06 mg/l, in the range of 1-36 mg/lt. The value increases with the value of 9.3 mg/l compared to the period of the previous season. In the rainy season (December) average TSS 11.94 in the range of 1-104 mg/l. The decrease in the rainy season due to the suspended material has been reduced by rainfall in the transition season.

5. Salinity

Salinity varied between seasons in Purworedjo. Measurement in rainy to dry transition (May) obtained average salinity of 0.0052‰, ranged 0.0009-0.0173‰. Salinity decreased during dry season (July) to 0.0037‰, in the range of 0.001-0.03‰. Deviation between the two seasons was 0.0015‰. Further measurement in dry to rainy transition (October) showed salinity of breeding places decreased slightly to an average of 0.0033 ‰, with a range of 0.0009-0.0173 ‰. As well as in rainy season (December) salinity has decreased by an average of 0.0023 ‰, with a range of 0001-0008 ‰. (Figure 3)

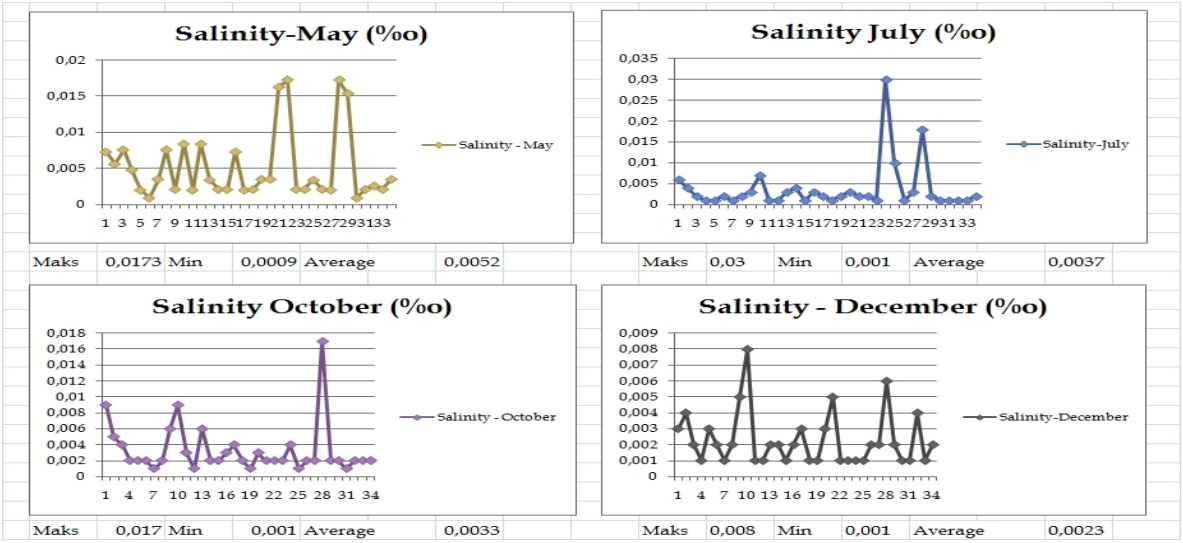

Figure 3 : variety of salinity of 4 survey periode

6. Dissolved oxygen (DO)

At the transition rainy to dry season (May) average DO was 3.7 mg/l, ranged 1-6.2 mg/lt. DO increased in the dry season (July) to 4.4 mg/l, ranged 2-6.8 mg/lt. There is a deviation of 0.7 between the two seasons. In transition of dry to rainy season (October) the average DO was 4.6 mg/l, ranged 2.1-6.6 mg/lt. The DO increased by 0.2. In rainy season (December) average DO was recorded at 5.2 mg/l, ranged 2.3-7.4 mg/lt. Deviation between transition and rainy season was 0.6 mg/lt.

7. Chloride (Cl)

Measurement in dry to rainy transition obtained average Cl concentration of 10.1 mg/l, ranged 1.7-30.8 mg/l. The average decreased to 6.15 mg/l in dry season, ranged 1.7-36.6 mg/l. Deviation of 3.95 was occurred between the two seasons. Further measurement in transition from dry to rainy season showed average of 7.8 mg/lt, ranged 1.7-40.9 mg/l. There was 1.65 difference between dry and transistion. Measurement in rainy season showed chloride concentrations declined by an average of 5.0 mg/l, ranged from 1.7-6mg/lt. Deviation of 2.8 occurred between the transition and the rainy season.

8. Electricconductivity (EC)

Our study showed an EC variations between seasons. Measurement in rainy to dry transition showed average EC 234.8 µmhos/cm, ranged of 91-604 µmhos/cm. In dry season average EC 249.41 µmhos/cm, ranged 57-567 µmhos/cm. There was 14.61 difference between transition dry to rainy with dry season. In dry to rainy transition EC increased the average of 279.41 µmhos/cm, ranged 126-488 µmhos/cm. Deviation between the periods was 30. In the rainy season EC decreased to 192.94 µmhos/cm, ranged 64-458 µmhos/cm. Deviation of 86.47 occured between the transition season and the rainy season.

9. Hardness

Our result showed hardness varied between seasons during the observation. Measurement in rainy to dry transition showed an average hardness of 88.18 mg/l CaCO3, with a range from 26.8-184.01 mg/l CaCO3. Measurement in dry season showed hardness increased to an average of 108.03 mg/l CaCO3, ranged 25.74-240.57 mg/l CaCO3, which was an increase of 19.85. Measurement in dry to rainy transition obtained hardness average of 116.68 mg/l CaCO3, ranged 24.88-254.52 mg/lt. Deviation compared to previous season was 8.08. Measurement during rainy season showed an average hardness of 81.27 mg/l CaCO3, ranged 28.86-197.99 mg/lt. Deviation occured was 35.41.

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Air quality (resting place)

1. Air temperature

There was variation of air temperature during seasons. On May we obtained an average air temperature 28.3ºC, ranged 23.5 to 31 ºC. At follow-up measurements On July temperatures decreased to 26.9 ºC, ranged 23-32 ºC. There was 1.4 ºC difference between rainy-dry transitions with dry season. On October the average air temperature was 29.1 ºC, ranged 26.5-33 ºC. Deviation was 2.2 ºC between the two seasons. On December average temperature was 26.29 ºC, ranged 22.5-30.5 ºC. The average temperature decreased 2.81 ºC (Figure 4).

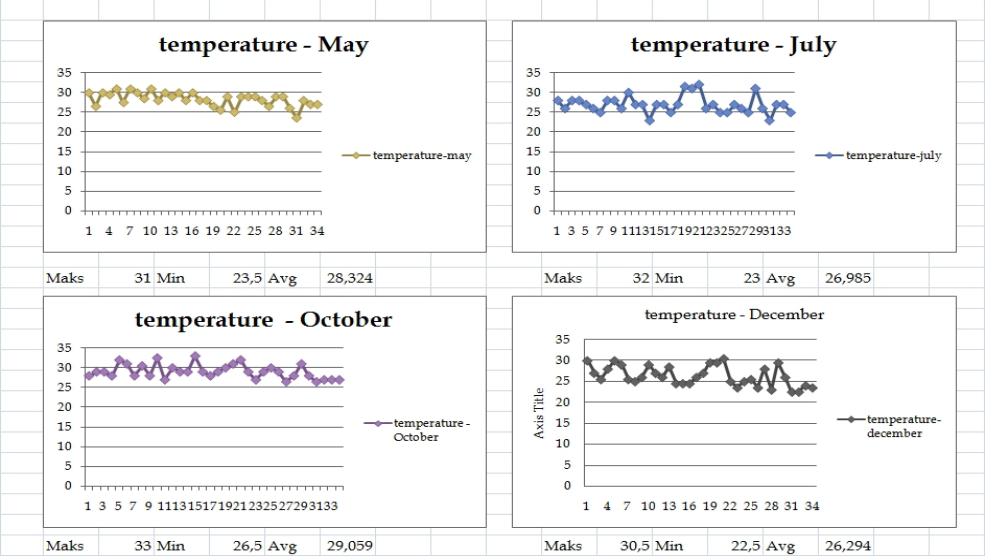


Figure 4. variability of air temperature of 4 survey periode

2. Humidity

Measurement on May found average relative humidity was 80.6%, ranged 71-96%. Measurement on July obtained an average humidity of 79.8%, ranged 61-89%. Humidity changed about 0.8%. Measurement on October obtained an average humidity of 68.41%, ranged 52-79%. The humidity changed 11.3% from previous season. Measurement on December obtained an average humidity of 86.3%, ranged 70-100%. This changed 11.4% from previous season.

3. Wind speed

Average wind speed in transitiondry-rainy season was 1.6km/h, ranged 1.08-2.88 km/h. In dry season the average wind speed was 1.86 km/h, ranged 1.08-3.96 km. The wind speed in dry season was 0.26 km/h faster. In transition dry-rainy season we obtained average wind speed of 3.42 km/h, ranged 1.25-8.57 km/h. This was 1.56 faster compared to previous season. In rainy season the average wind speed was 2.35 km/h, ranged 1.05-5.56 km/h. This was 1.07 km/h slower compared to the previous season.

Bionomic of malaria vector

Mosquito catching was conducted at 06:00 pm-06:00 am. Air temperature decreased from 26.5 ºC at 06:00 pm to 23 ºC at 03:00 am, after that the temperature was relatively constant until 05.00 am. *An. balabacensis* have intermittent movement pattern, found from 08:00-09:00 pm, and found again in low density at 11:00 pm to 01:00 am. *An. aconitus* was consistently found throughout 06:00 pm to 06:00 am, only paused between 04:00-05:00 am. *An. barbirostriss* was started to catch at 07:00 pm until 00:00 am, the activity continued at 00:00-05:00 am. *An. vagus* was a dominant species, was found throughout the night, and had 2 density peaks, at 08:00-09:00 and 00:00 am. *An. anulari s*was started to catch at 10:00 pm until 05:00 am in a medium density. Activity of *An. kochi* was started at 08:00 pm until 06:00 am with a relatively constant density. *An. maculatus* had activity around 10:00 pm-01.00 am. *An. indifinitus* found at 07:00-08:00 pm, when all other species were not found (Figure 5).

Uraian		18 -19	19 - 20	20 -21	21 - 22	22 - 23	23-24	24 - 01	01-02	02-03	03-04	04-05	05-06
Suhu	° C	26,0	25,5	25,0	24,5	24,5	24,5	24,0	23,5	23,5	23,0	23,0	23,0
	° F	78,8	77,9	77	76,1	76,1	76,1	75,2	74,3	74,3	73,4	73,4	73,4
1	35												
2	34												
3	33												
4	32												
5	30												
6	29												
7	28												
8	27												
9	26												
10	25												
11	24												
12	23												
13	22												
14	21												
15	20												
A. balabaensis				2			1	1					
A. aconitius		1	1	3	4	2	2	2	1	2	2		2
A. barbirostres			1		1			1	3	3	3	2	
A. vagus		3	17	23	1	6	2	17	6	12	19	3	3
A. anularis						4	3	3	3	1	2	2	
A.kochi				2		1	1		2	5	2	1	1
A. maculatus						2		2					
A.indifinitus			1										

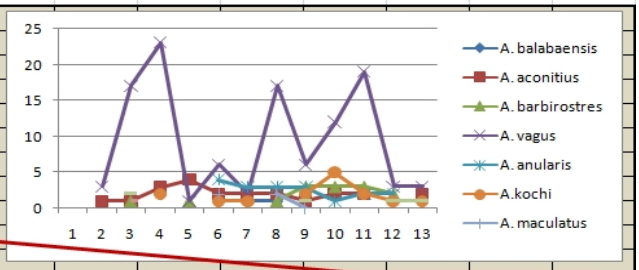


Figure 5 : Bionomic of Anopheles of endemic area of Purworejo

Analysis of environmental variabilityand vector density

Discriminant factor analysis generated variables that affect the interests of the vector density on May were TDS (0.737), chloride (0.943), turbidity (0.733), air temperature (0.837), humidity (0.578), and wind speed (0.799). In the dry season (July) the discriminant variableswere TDS (0.903), chloride (0.889), salinity (0.949), air temperature (0727), humidity (0.792), and wind speed (0.894). Dry to rainy transition season had TDS (0.682), salinity (0.648), hardness (0.755), conductivity (0.523), DO (0.867), air temperature (0.726), humidity (0.777), and wind speed (0,665) as discriminant variables. In the rainy season the variables consisted of chloride (0.854), turbidity (0.319), hardness (0.705), DO (0.817), pH (0.796), air temperature (0.626), humidity (0.721) and wind speed (0,678).

Table 3 showed habitat for each species. *An. balabaensis* found in areas with an altitude of 50-500 mpdal, pH 4.3-8.3, DO 1.3-7.4, conductivity (44.78-604), hardness 24.88-206.91), salinity (0001-0.018), turbidity (1-48), chloride 1.7-36.6), TDS 39-239. Habitat of *Anopheles aconitus* was areas with altitude 0-750 mpdal, pH 4.3-8.5, DO 1.7-7.4, conductivity 57-488, hardness 24.88-254.52, salinity 0.0009-0.018, turbidity 1-58, chloride 1.7-40.9, and TDS 37-890. Distribution of species base of elevation showed at figure 6.

Table 3. Habitat characteristic of species Anopheles

No	habitat characteristic	malaria species								
		An.balabaensis	*An.aconitus*	*An.barbirotres*	*An.vagus*	*An.anularis*	*An.kochi*	*An.maculatus*	*An.indifinitus*	*An.subpictus*
1.	elevation, mdpal	50-100	0-750	0-500	0-800	350-500	0-500	50-500	150-400	50-300
2.	breeding place quality									
	TDS (mg/lt)	39-239	37-890	54-890	37-890	54-229	37-230	42-310	55-230	51-268
	Chlorida (mg/lt)	1,7-36,6	1,7-40,9	1,7-30,8	1,7-40,9	1,7-20	1,7-30,8	1,7-29,9	2-30,8	2-10
	turbidity (NTU)	1-48	1-58	1-58	,1-58	1-48	1-48	,1-48	1-31	2-31
	salinity (‰)	0,001-0,018	0,0009-0,018	0,0009-0,0163	0,0009-0,03	0,0009-0,009	0,0009-0,0163	0,0009-0,03	0,001-0,0163	0,0009-0,0035
	hardness (mg/lt)	24,88-206,91	24,88-254,52	31,84-172,26	24,88-254,52	44,78-119,18	24,88-175,12	28,86-240,57	48,24-152,51	42,21-254,52
	conductivity (µmhos/cm)	44,78-604	57-488	91-481	57-488	109-471	57-481	64-481	116-481	106-488
	DO (mg/lt)	1,3-7,4	1,7-7,4	1,7-7	1-7,4	2,7-7	1,9-6,9	1,7-7,4	1-6,5	2,1-5,9
	pH	4,3-8,3	4,3-8,5	5,6-8,5	4,3-8,5	5,9-8,3	4,3-8,4	5,6-8,5	6,5-7,5	6,3-7,5
3.	resting quality									
	temperature (°C)	22,5-31	22,5-33	24,5-33	22,5-33	24,5-32,5	23,5-32	22,5-32,5	25,5-32	25.5-32
	humidity (%)	61-90	52-89	52-93	52-95	60-89	55-96	55-95	61-88	61-88
	wind velocity (km/h)	1,08-5,56	1,08-7,4	1,05-7,4	1,05-8,57	1,05-4,48	1,05-7,4	1,05-7,4	1,08-5,56	1,08-4,36

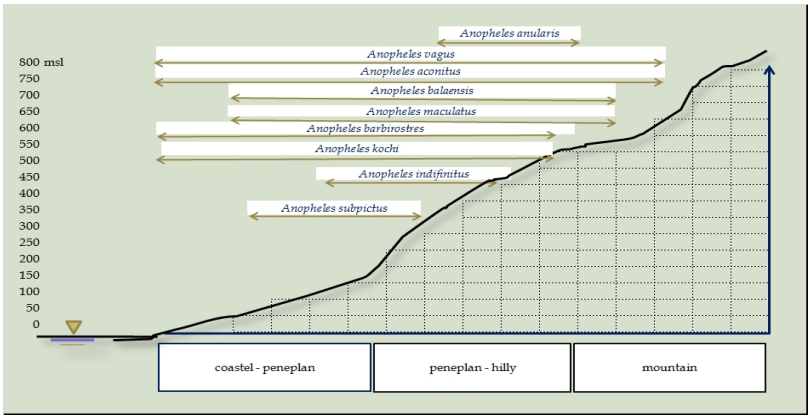


Figure 6. Distribution of species base of elevation at Purworejo

Our results showed habitat suitability index (HSI) experienced dynamic variation between seasons. In rainy to dry transition, regions were HSI medium (29%), high (9%), very high (3%), the corresponding overall (41%). HSI in dry season was medium (35%), high (21%), very high (9%), which corresponds to 65% overall. HSI in rainy to dry transition was medium (24%), high (24%), very high (32%), 79% overall. In rainy season the HSI was medium (38%), high (15%), very high (26%), 79% overall (Figure 7).

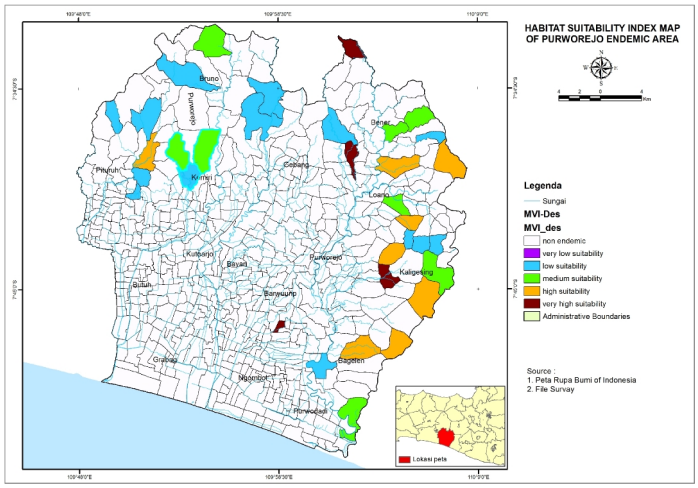


Figure 7. Map of Habitat Suitability Index of Purworejo Endemic Area

DISCUSSION

Malaria remains a health problem in Indonesia. Various factors conical and support each other (convergent) on malaria transmission [13]. Vector of malaria as one component of malaria transmission has ecological system [18]. Every landscape on earth has experienced characteristics and ecological processes of life on it [19,20]. Each region with its characteristic habitat for living beings has always experienced the dynamics of natural processes [21-23]. Weather changes that occur globally give life extensively, both to humans and disease patterns [24]. Malaria is a disease that is affected by global climate change [3,25-30].

Malaria habitat shifts in a wider space. Altitude regions that have not previously found malaria, it is currently found malaria and it vectors, Anopheles [31.32]. Mapping in malaria endemic areas, such as Purworejo, is a strategic step in the control of malaria. Knowing the characteristics of the region and spesific habitat was the key to controlling malaria vectors [33].

Our results showed *Anopheles* species experienced the dynamics of each season, consisted of 9 species in rainy to dry transition, 8 species in dry season , 8 species in dry to rainy transition, and 7 species in rainy season. This proves that the *Anopheles* species have dynamics over time from one season to another season in a year.[34]. The changes are caused by a variety of factors. One of which is the change in quality of habitat [35]. Our study showed habitat changes, which were mainly from the breeding place.

Characteristics of the breeding place changed in every season. In this study, we proved that acidity, turbidity, TDS, TSS, salinity, dissolved oxygen, chloride, conductivity, and hardness were all changed in accordance with seasons. Air quality also changed during observation, consisted of temperature, humidity, and wind speed. According to the law of tolerance Shelford, living beings will grow and develop in accordance with the betas tolerance [21]. Environmental characteristics provide materials and energy as a source of nutrients for optimal living beings to limit growth. At concentrations of less or excess of matter and energy it is precisely as a barrier to growth [19,22]. Each region has environmental quality, water and air, which is influenced by the interaction geosphere, biosphere, lithosphere, hydrosphere and atmosphere [23,36].

Every living being will choose a place to live (habitat) in accordance with the need to grow and develop optimally [37]. If there is a change there will be a migration of habitat, adaptation, mutation or extinction [38.39]. The distribution of malaria vectors in Purworejo is in accordance with nature of every living being. Living being always attempts to locate the material and the energy of the habitat in which to grow and develop optimally [40]. *An. vagus* and *An. aconitus*live in a range of 0 mdpal (coast) to 850 mdpal (hills) because both species have a high tolerance to grow and thrive. Tolerance is not shared by other species, so the species is more limited range distribution [41].

Distribution of Anopheles in Purworedjo according with height range region forms a particular pattern. *An. subpictus*, *An. anularis* and *An. indifinitus* have a limited distribution range. This result is consistent with studies in other parts of Indonesia [42-44] and parts of Africa, Brazil or Asia [32,45-51], indicating the existence of a certain range of the distribution of Anopheles species.

Environment as a limiting factor is for Anopheles was water quality (as a place to breed) and air quality (as a place to rest) [52]. Cluster and discriminant analyses revealed main parametersof environmental had seasonal dynamics. Season cycle as discriminant was TDS (0.737), chloride (0.943), turbidity (0.733), air temperature (0.837), relative humidity (0.578), wind speed (0.799), salinity (0.949), hardness (0.755), conductivity (0.523), dissolved oxygen (0.867), and pH (0.796). Our study suppported theory that parameters of water and air qualities experienced seasonal dynamics throughout the year [23,53]. Rainfall and surface water runoff will dissolve the chemical elements of rock and soil, thereby providing direct effect on changes in water quality parameters [54]. Air quality parameters are influenced by the movement of the sun that affects climate change. Topographically each region has distinct regional characteristics [36].

Dynamics of environmental quality (water and air) that occurs in each region will affect the breeding cycle of the vector [55]. Mosquito cycle of egg, larva, pupa, and adult mosquitoes is affected by the water composition suitable for growth. At good composition, accompanied with suitable energy and material for breeding, vector will occus in hight density [56]. Our results from 34 locations confirmed the dynamics of environmental quality and the dynamics of the vector density. The composition of air and water quality on every land, form a specific habitat for malaria vectors.Habitat suitability index (HSI) is an index that gives an overview about the suitability of any area for growing and breeding of malaria vectors [40]. Our result showed each species of Anopheles has specific environmental characteristics needed to breed. For example, *An. aconitus* and *An. balabacencis* have different need for altitude, pH, DO, conductivity, hardness, salinity, turbidity, chloride, and TDS. Similarly, other species also has a specific habitat. Our study showed similar results with other studies in Asia (Bangladesh and Vietnem) and Sudan, which also showed characteristics of a region served as determinant of malaria vectors [48,58,59,60]. Our result also showed HSI in Purworejo experienced dynamics in every season. This was similar to previous studies [26,46,64,70].

CONCLUSION

The environmental had dynamic characteristics and effect on life cycle ofmalaria vector. Species found during one year observation were *An. balabacensis, An. barbirostris, An. vagus, An. maculatus, An. aconitus, An. kochi, An. indifinitus, An. anularis, An. supictus*. ELISA test results showed *An. maculatus* and *An. balabacensis* served as malaria vectors. Environment as dynamics parameters for Anopheles are TDS (0.737), chloride (0.943), turbidity (0.733), salinity (0.949), hardness (0.755), conductivity (0.523), dissolved oxygen (0.867), pH (0.796), air temperature (0.837), relative humidity (0.578), and windspeed (0,799). Suitable habitat for Anopheles in Purworedjo based HSI was as follow: rainy to dry transition (41 %), 65% of the dry season, the dry yo rainy transition 79 % and rainy season 79 %. Further research needs to be done to measure the environment quality and vector density in other regions in Indonesia, so it can be used for mapping of areas with high HSI, as malaria vector control

REFERENCES

1. Witular R, Perubahan Iklim Implikaisnya Bagi Indonesia, Laporan Tahunan, Jakarta, Dewan Nasional Perubahan Iklim, 2011, p.25-35
2. United Nation Development Program de Indonesia (UNDP), Climate Risk Management an Integration Approach For Climate Change Adaptation and Disease Risk Reduction in Indonesia, Jakarta, Human Development Report, 2009, p.78-98
3. IPPC a,b,c Ecosystem, Human Health, Intergovernmental Panel on Climate Change,Cambridge, Cambridge University Press, 2007, p : 112-128
4. Anyamba A, JP Chretien, Developing Global Climate Anomalies Suggest Potential Risk, International Journal Of Health Geographics, 2006, 5(60):49-69
5. Mc.Michael A.J, S Hales, Climate Change and Human Health: Present and Future Risk, Lancet, 2006, 367 (9513: 859-869)
6. Sutrisno B, Pengantar Metode Epidemiologi, Jakarta, Dian Rakyat, 1994, p : 12-18
7. Aswar. A, Pengantar Ilmu Kesehatan Lingkungan, Jakarta, PT. Mutiara Sumber Widya, 1986
8. Fox JP, Hall CE. Epidemiology Man and Diseases, New York, MacMillian Co, 1970
9. WHO, Mannual of Practical Entomology in Malaria, Geneva, WHO, 1975
10. IOM, Understanding the Environmental Human Health and Ecological Connections, Wasington DC , the National Academic press, 2008, p : 10-18
11. Dinas Kesehatan Purworejo, Laporan Malaria Tahunan, Purworejo, tidak di terbitkan, 2011
12. Fahmi AU. Manajemen Penyakit Berbasis Wilayah, Jakarta:UI Press,2007, p.38-42
13. IOM, Microbial Threats to health : emergence, detection and response, Washington, DC: The National Acadeic Press, 2003, p : 48-62
14. Sukowati S, Perilaku vektor malaria di Kabupaten Purworejo, Laporan penelitian, Jakarta, Litbangkes, 2011
15. Mursid, Malaria Vulnerability Index Untuk Manajemen Risiko Dampak Perubahan Iklim Global TerhadapLedakan Malaria di Indonesia, Vektora Jurnal Vektordan Reservoar Penyakit, BBPPVRV, Salatiga, 2011, Vol III No 1, 54-80

16. Manguin S, Garros C, Bionomics, taxonomy, and distribution of the major malaria vector taxa ofAnophelessubgenusCelliain Southeast Asia, Infection, Genetics and Evolution, 2008, (489–503)
17. National Research Council, Under the weather : climate, ecosystem, and infectious diseases, Wasington DC, National Academy Press, 2001
18. Castro CM, Tsuruta A, Community-Based Environmental Management For Malaria, Malaria Journal, 8:57, 2009, doi : 10.1186/1475-2875-8-57
19. O'Riordan T, Environmental Science for Environmental Management, London, Longman Science&Technical, 1995
20. Paaijmans K, Susan S I, Matthew B T, Relevant Microclimate For Determining The Development Rate Of Malaria Mosquitoes And Possible Implications Of Climate Change, Malaria Journal , 2010, Vol 9:196
21. Odum T, Basic of Ecology, New York, John Wiley&Sons LTD, 1988
22. Melinda SM, Michael E, Medical Geography, Third Edition, New York, The Gulford Pres, 2010
23. Beroya AM, Mengenal Lingkungan Hidup, Jakarta, Rineka Cipta, 2000
24. Brewer R, The Science of Ecology, Second Edition, New York, Saunders College Publishing, 1993
25. Huggett JR, Geoecology An evolutionary approach, New York, Routledge, 1995
26. Thomson MC, Palmer TN, Malaria early warnings based on seasonal climate forecasts from multi-model ensembles, Nature, 2006,Vol 439
27. Bernard M, Deadly Diseases and Epidemic Malaria, Second Edition, , New York Chelsea House, 2009
28. Craig and David, A Climate-based Distribution Model of Malaria Transmission in Sub-Saharan Africa, South African Medical Research Council, South Africa, Parasitology Today, 1999, vol. 15, no. 3
29. David AR, Margaret AH, Eileen R, Global Climate Change and Extreme Weather Event, New York, The National Academic Press, 2008,
30. Dixon G.P, Climate Change and Human Health, special issue of International Journal of Environmental Research and Public Health, 2010,Vol 5 : 78-91
31. Lioubimtseva E, Henebry GM, Climate and Environmental Change in Central Asia: Impact, Vulnerability, Adaptation. Journal of Arid Environments2009;4:44-62
32. Muthers S, Matzarakis A, Climate Change and Mortality in Vienna, A Human Biometeorological Analysis Base On Regional Climate Modeling, Environmental Research and Public Health, 2010
33. Adlaoui E, Faraj C, Bouhmi EM, Mapping Malaria Transmission Risk in Northern Morocco Using Entomological and Environmental Data, Malaria Research and Treatment Volume 2011
34. Ageep TB, Cox J, Hassan MM, Spatial And Temporal Distribution Of The Malaria Mosquito Anopheles Arabiensis In Northern Sudan: Influence Of Environmental Factors And Implications For Vector Control, Malaria Journal, 2009, Vol 8:123
35. Tonnang HE, Kangalawe R, Yanda P Z, Predicting And Mapping Malaria Under Climate Change Scenario : The Potential redistribution of Malaria Vector in Africa, Malaria Journal, 2010,9-111
36. Sven EJ, Robert C, Ecological Indicators For Assessment Of Ecosystem Helath, New York,CRC Press, 2005

37. Valeri O, Piere D, Marc C, The Anopheles dirus complex : Spatial Distribution and Environmental Drivers, Malaria Journal, 2009, 6:26
38. Okay TR, Boundary Layer Climate, Second Edition, London, Routledge, 1987
39. Souza D, Kelly L, Environmental Factors Associated with the Distribution ofAnopheles gambiaes.s in Ghana; an Important Vector of Lymphatic Filariasis and Malaria, PlosOne, 2010, Volume 5 | Issue 3 | e9927
40. Ayala D, Costantini C, Habitat Suitability And Ecological Niche Profile Of Major Malaria Vectors In Cameroon, Malaria Journal, 2009,Vol : 8:307
41. Farina. A, Principles and Methods in Landscape Ecology, New York, Chapman & Hall, 1998
42. Mardihusodo SJ, Malaria Status Kini dan Pengendalian Sp Vektornya untuk Abad XXI, Universitas Gadjah Mada, Yogyakarta, 1999
43. Baroji, Buwono DT, Penelitian Spesies Malaria Kabupaten Purworejo, Salatiga, Buletin Kesehatan, BBP2VRP, 1992
44. Pat Dale, Ndon, Malaria In Indonesia: A Summary Of Recent Research Into Its Environmental Relationships, Southeast asian Journal Tropmed Public Health, 2005, Vol 36 No. 1 January 2005
45. Utarini A, Evaluation Of the User-Provider Interface in Malaria Control Programme : The Case of Jepara District, Fakultas Kedokteran Universitas Gadjah Mada, Yogyakarta, 2002
46. Widayani, Pemanfaatan Remote Sensing untuk Deteksi Penyebaran Malaria di Kabupaten Purworejo, Laporan Penelitian, Perpusatakaan Geografi UGM, Yogyakarta, 2010
47. Li li, Bian L, A Studi Of the distribution and abundance of the adult malaria vector in western Kenya highland, International Journal of Health Geographics, 2008, 7:50 doi : 10.1.1186/476-072X/7-50
48. Garos C, Cam V NG, Ho D T, Distribution Of Anopheles in Vietnam, With Particular Attention to Malaria Vectors Of The Anopheles Minimus Complex, Malaria Journal, , 2008, Vol : 7:11
49. Diego A, Carlo C, Kenji O, Habitat Suitability and Ecological Niche Profile Of Major Malaria Vectors in Cameron, Malaria Journal, 2009, Vol : 8:307
50. Marinete M, Nazare M, Voa, Malaria Vectors, Epidemiology, And The Re-Emergence Of Anopheles Darlingi In Bele´ M, Para´ Brazil, Journal Of Medical Entomology 2003, Vol 40,
51. Obsomer V, Defourny P, The Anopheles dirus complex: spatial distribution and environmental drivers, Malaria Journal , 2007,Vol 6:26
52. Kulkarni MA, Desrochers ER, High resolution niche models of malaria vector in nothern Tanzania : A new capacity to malaria risk, Plos One, 2010, 5(2) e9396 doi :10.1371/jounal plosone.0009396
53. Sousa D, Louise KH, Environmenal Factor Associated With The Distribution of Anopheles gambiae in Ghana : an importan vector of Malaria, Plos One, 2010, Volume 5, Issue 3, e9927
54. Roklov J, Forsberg F, The effect of high ambient tempertaure on elderly population in three region Sweden, International Journal Environmental Res. Public Heakth, 2010, 7:2607-2619 doi : 10.3390/ijerph.7062607
55. Strahler A, Strahler A, Physical Geograeteksphy Science And System Of The Human Environment, , New York, John Wiley&Sons Inc, 1997

56. Obsomer V, Defourny P, The Anopheles dirus complex : spatial distribution and enviroenmental drivers, Malaria Journal, 2008, 6:26, doi : 10.1186/1475-2875-6-26
57. Lioubimtseva E, Henebry GM, Climate and environment change in arid Central Asia : Impact, Vulnerability and Adaptation, Journal of Arid Environments, 2009, 73 (2009) 963-977, doi : 10.1016/j.jaridenv.2009.04.022
58. Maguire DJ, Tuti S, Endemic Coastal malaria in The Thousand District Jakarta, Tropical Medicine and International Health, 2005, volume 10 no 5 PP 489-496
59. Khrisnamoorthy K, Jambulingam, Altered environment and risk malaria outbreak in South Andaman, India, Malaria Journal, 2005, 4:32 doi:10.1186/1475-2875-4-32
60. Subbarao KS, Anopheline Species Complexes in South-East Asia, WHO, New Delhi, 1998
61. Stanley M, Fredrick P, Vector Born Diseases The Environmental Human Health and Ecologycal Connection, Workshop Summary, New York, The National Academic Press, 2008
62. Tanga MC, Ngundu WI, Judith N, Climate Change and Altitudinal Structuring of Malaria Vector in South Western Cameroon : Their Relation to Malaria Transmission, Transaction of The Royal Society Of Tropical Medicine and Hygiene,2010, Vol 104,453-460
63. Tella BA, Cox J, Hasan MM, Spatial and Temporal Distribution of Malaria Mosquito Anopheles arabiensis in Nothern sudan : In Influence of Environmental factors and implication for vector control, Malaria Journal, 2009, 8 : 123 doi :10.1186/1475-2875-8-123
64. Ubudul H, Masahiro H, The Role of Climate Variability in the Spread of Malaria in Bangladesh Highlands,PlosOne , 2010, 5(12):e14341. doi:10.1371/journal.pone.0014341
65. Wernsdorfer HW, Global Challenges of Changing Epidemiological Pattern of Malaria, Actatropica Elsevier, 2011, 06.014
66. Dambach P, Sie A, Using high spatial resolution remote sensing for risk mapping of malaria occurrence in the Nouna district, Bukina Faso, Global Health Action 2009, 2009, doi : 10.3402/gha.v2io.2094
67. Eisele PT, Keating J, Swlam C, Lingking field base ecological data with remoteley sensed data using a geographic information system in two malaria endemic urban area of Kenya, Malaria Journal, 2003, 2:44
68. Gillian H. S, Beyond Temperature And Precipitation: Ecological Risk Factors That Modify Malaria Transmission, Acta Tropica, 2009,Vol 116 : 167-172
69. Machault V, Vignolles C, Pagès P, Spatial heterogeneity and temporal evolution ofmalaria transmission risk in Dakar, Senegal, according to remotely sensed Environmental data, Malaria Journal,2010, Vol 9: 252
70. Phimpraphi W, Paul RE, Yimsamran S, Longitudinal study of Plasmodium falciparum and Plasmodium vivax in Karen population in Thailand, Malaria Journal, 2008, Vol. 7:99
71. Rokotomanana F, Ratovonjato J, Randremanan RV, Geographical nd Environmental to Urban Malaria in Antananarivo Madagascar, BMC Infectious Diseases, 2010, 10:173
72. Reiter P, Global Warming and Malaria : Knowing The Horse Before Hotching The Cart, Malaria Journal, 2008, 7 (Suppl), doi:10.1186/1475-2875-7S1-S3

PREFERENCES OF *Aedes aegypty*IN HACTCHING EGGS ON OVITRAPS WITH SEVERAL TYPES OF ATTRACTANT

Akram Bugis, Martini, Retno Hestiningsih, Sri Yuliawati
Epidemiology and Tropical Disease Department
Public Health Faculty-Diponegoro University
Email: tinihen65@yahoo.co.id

ABSTRACT

Background: A method to control *Aedes aegypti* in order to decrease the density of the vector is the use of the egg trap (the ovitrap). Attractant is usually used together with the ovitrap as attractant does not create a risk because of inhaling dangerous chemical contained in insecticide and fogging. The aim of this research was to identify the differences of the *Aedes aegypti's* preferences to hatch on the ovitrap using different types of attractants.

Method: This study was an experimental laboratory research with which variables measured were the number of *Aedes aegypti's* eggs trapped in the ovitrap and the attractants (yeast solution, hay infusion, and rain water.) Each unit of the experiment consisted of 25 blood fed *Aedes aegypti*. The number of eggs was calculated after five days. The number of replication of each unit was nine.

Result: ANOVA analysis showed that there were significant differences on the number of *Aedes aegypti's* eggs in the ovitrap using different attractants at 0.05. The average number of eggs in the ovitrap is, respectively, 669.1 eggs in the hay infusion, 297.3 eggs in the rain water, 114.4 eggs in the yeast solution, and control positive (+) 314.1. The highest number of *Aedes aegypti's* eggs in the ovitrap was the one uses hay infusion compare to yeast solution (p=0.000), rain water (p=0.000), and control (p=0.000).

Conclusion: Hay infusion was most effective than yeast solution and rain water attractant for oviposition of gravid *Aedes aegypti*.

Keywords: Ovitrap, hay infusion, attractant, *Aedes aegypti*

BACKGROUND

Aedes aegypti is a vector of several serious diseases exposed to human, such as, malaria, encephalitis, "yellow fever", dengue fever, filariasis, and arbovirus [1]. Among the most important vectors causing dengue fever; *Aedes aegypti, Aedes albopictus,* and *Aedes scutellaris; Aedes aegypti* is the major vector [2].The number of cases of dengue fever in Indonesia tends to increase from year to year. The increase of the dengue fever in many cities in Indonesia is caused by the difficulty to control the disease infected by *Aedes aegypti* [3,4].

Until today, there has no vaccine found to prevent dengue hemorrhagic fever [5,6]; so that, the management applied to these diseases is primarily aimed to cut off the infection spread out by controlling their vectors. The effort to control dengue fever's vector that has been carried out by all countries and endemic area proved to be not effective, unsustainable, and failing to break up the chain of infection [7].

The most effective precaution is by obliterating the mosquito's breeding sites through draining containers, to cover up tightly collecting and saving water, and burying unusable materials that might become the breeding place for *Aedes aegypti.* Moreover, the use of larvacide in wiping out the mosquito is also effective [8]. Some studies claimed that the knowledge of the society about the way to destroying *Aede saegypti* remains lacking [9]. As a result, restrain using chemical substances is popular in community and programs introduced to them although the use of insecticide to control dengue fever has both advantages and disadvantages [10]. Program to control vector implemented in many countries, including Indonesia, has not been successful because it depend on the application of fogging. This method costs 5 billion rupiahs per year, and it might develop resistance to the vector due to the inappropriate dosage use [11].

Recently, the use of alternative method to decrease the density of *Aedes aegypti* is considered significant in some countries, especially the use of the ovitrap. Unlike insecticide and fogging, attractant, mostly apply together with the ovitrap, does not contains dangerous chemical substances [12]. Mosquito can detect carbon dioxide (CO_2) from respiration of human, animal, and microorganism metabolism process. Mosquito approaching those sources of CO_2 will be trapped. The higher the volume of CO_2 and other attractants the easier the mosquito detects them. This caused chances of the mosquito to come closer increase [13].

The ovitrap modified using ten percent of hay infusion increases the number of eggs trapped into it eight times higher [14.15]. This attractant that produce CO_2, ammonia, and octane proves to be influencing the nerve system of mosquito's smell [16,17]. Meanwhile, fermented cassava (*Saccharomyces cereviceae*) produces methane gas, CO_2, small amount of other gases such as H_2S, and water [18]. Moreover, rain water is also suspected to be containing of more gases than soil water does, especially CO_2 and O_2 [19]. The objective of this study is to identify the different preferences of *Aedes aegypti* in hatching in the ovitrap using different type of attractants.

METHOD

Sample of this research was female blood fed *Aedes aegypti* collected from a survey of mosquito larvae and the ovitrap assembled in the field. At the rearing until maturity stage, they were fed by guinea pig blood, and the research was conducted in entomology laboratory of the Public Health Faculty of Diponegoro University. Research was carried out by conducting preliminary test to determine the concentration of yeast solution (0.5 grams, 1 gram, 2 grams, and 3 grams) dissolving in 200 milliliter of aqua distillation. Preliminary research showed that the concentration of 0.5 grams yeast dissolved in 200 milliliter (2.5 grams/l) was used to be an attractant. Other treatments were 10% hay infusion, and drinking water as positive control.

Variables of this research were number of eggs and larvae of *Aedes aegypti* trapped in ovitrap, and the type of attractants. This study was a true experimental research using pre-post test with control group design. The test to the type of attractants preferred by hatching *Aedes aegypti* was conducted by putting 25 blood fed *Aedes aegypti* into the 50 cm^2 cage with the ovitrap and the tested attractant for 5 days.

Number of eggs in the ovitrap with the attractants was calculated to be analyzed further. During the experiment, the room temperature was measured in the morning

(08:00-10:00 a.m.) and in the afternoon (01:00-03:00 p.m.). In addition, the temperature and pH of the attractant media was also noted. The experiment was conducted with 9 replications, and the data collected was analyzed using one way ANOVA.

RESULT

The highest average number of the pre-mature *Aedes aegypti* in the ovitrap using different type of attractants was 669.1 found in the ovitrap with 10% of hay infusion. The least attractant attracting mosquito to hatch was 114.4 pre-mature *Aedes aegypti* found in the ovitrap with yeast solution of 2.5 grams/lt. The following Table 1 shows the number of larvae found in the ovitrap after 5 days observation; the highest number of larvae was found in the ovitrap with 10% of hay infusion.

Table 1: Number of pre-mature *Aedesaegypti* (eggs and larvae) found in the ovitrap using different type of attractants.

Replication	The number of pre-mature *Aedesaegypti* based on the type of attractants							
	Yeast solution of 2.5 grams/ liter		Hay infusion of 10%		Rain water		Control	
	Eggs	Larvae	Eggs	Larvae	Eggs	Larvae	Eggs	Larvae
1	43	0	642	0	450	0	275	0
2	282	0	573	0	314	0	318	0
3	67	0	635	3	297	2	357	0
4	96	0	692	0	283	0	342	0
5	174	0	570	2	442	0	301	0
6	58	0	921	0	169	0	189	2
7	54	0	586	5	201	0	463	0
8	37	0	727	2	320	1	358	2
9	219	0	661	3	197	0	217	1
X	114.4 (a)		669.1 (c)		297.3 (b)		314.1 (b)	

Remark: the same letter following the average number of pre-mature (eggs and larvae) shows insignificant differences α=0.05 using Least Significant Different (LSD) Test

The test result using ANOVA (One Way Classification) shows that the value of $p<0.0001$; it means that there is a significant difference on the average number of the eggs of *Aedes aegypti* base on the type of attractants. Furthermore, LSD test shows that the hay infusion attractant attracts female *Aedes aegypti* to hatch compare to yeast solution (p=0.0001), rain water (p=0001), and control (p=0001); however, the result of the rain water attractant and control is p=0.720 (insignificant difference)

DISCUSSION

The significant difference of attractant using 10% of hay infusion to attract *Aedes aegypti* to hatch takes place; while, the attractant using 2.5 grams/liter of yeast solution seems to be not attracting *Aedes aegypti* to hatch because, physically, the feature of this

water was turbid. *Aedes aegypti* tend to hatch in the clean water and puddle water [20]. *Aedes aegypti* has a habit to breed in the puddle clean water; naturally or man-made one. The places that *Aedes aegypti* prefers are bathtub, abandoned used tire, used materials exposed to rain, and other places that rain water is stay in [21-25]. Research conducted by Phontas (2012) supported this finding. He claimed that the number of *Aedes aegyptis'* larvae is strongly affected by the degree of the turbid water and the light intensity. The higher the turbid water and the light intensity, the less the number of *Aedes aegyptis'* larvae is found [26].

Hay infusion is significantly the one that is preferred by *Aedes aegypti* to hatch. Hay infusion proved to produce CO_2, ammonia, and octenol that influence the nerve system of smell of the mosquito [14,15]. The ammonia and CO_2 in the hay infusion produce a specific smell that functions as an attractant to the mosquito. This specific smell is captured by mosquito's antenna that has a sensilla which has one or more bipolar smell nerve known as ORNs (Olfactory Receptor Neurons). ORNs are located in the tip of dendrite and of also that function to detect chemical substances. This sensory nerve transmits chemical impulse as electrical responds by carrying smelling information from perifer to lobus antenna as the first terminal in the brain. After entering the sendillum through cuticle pore, the smell molecule goes through lymph liquid to dendrite. Most of the smell molecule is easily evaporated and relatively hydrophobic. The smell tied up to OBPs (Odorant Binding Proteins) goes through lymph liquid. Not only is a carrying, OBPs also works to dissolve the smell molecule and function as smell information selector. When complex OBPs reach the membrane of dendrite, the smell will be tied up to trans-membrane receptor and it will be transferred to the surface of the intracellular membrane. Lastly, this electrical impulse will be delivered to the higher level of the center of the brain and it will be integrated to produce an appropriate behavior responds [27].

The number of *Aedes aegypti*'s larvae found in the ovitrap using hay infusion was bigger than the control. The number of *Aedes aegypti*'s eggs trapped in that ovitrap was higher than that of in the control; therefore, the chance of the eggs to hatch to be larvae stadium was bigger. The hatching reaction is caused by stimulation of the central nervous system of the larvae in the eggs. The hatching process is also probably caused by the crowded eggs [28].

The different level of acidity was found in the different attractants. The acidity of the attractant using 10% of hay infusion and the control was pH 7.0, the rain water' attractant was pH 6.5, and the pH of the yeast solution was 5.0. The mosquito larvae was mostly found in the neutral pH of 7.0 [15].The acidity (pH) of the cassava fermented water attractant changed to be lower (acid) of pH 5.0 because fermentation process was taken place in the ovitrap that caused the decrease of the pH in the ovitrap using yeast solution. This phenomenon does not support the *Aedes aegypti* preference that tends to hatch in the water with pH 7.0 (neutral) [29] or the development of the larvae to become mature ones [30].

The environment temperature during the experiment was $27.5^{-}28.8^{0}$ C (10:00 a.m.-03:00 p.m). Generally, the preferable temperature for *Aedes aegypti* to lay their eggs is $20^{-}30^{0}$ C. At the temperature of 30^{0} C the eggs will be hatched in 1 day to 3 days, and at the temperature of 16^{0} C the eggs will be hatched within 7 days [31]. The humidity measured was 73-75%; while, the preferable humidity for mosquito environment is 70-89% [32]. The fact shows that the temperature and the humidity in the laboratory during

the experiment did not affect the life cycle of the *Aedes aegypti* especially during the oviposition period.

CONCLUSSION

Attractant using hay infusion for preferable to *Aedes aegypti* to lay their eggs compare to other attractants (the using yeast solution and rain water). The average number of eggs in the ovitrap using hay infusion attractant is 667.4, rain water attractant is 297, and yeast solution attractant is 114.4.

Further study on the use of yeast solution with 0.5 grams concentration dissolved in 200 milliliter aqua distillation needs to done.

REFERENCES

1. Ndione, R.D., Faye O., Ndiaye M., Ndiaye A., and Afoutou J.M.2007. Toxic effect of neem products (Azadirachta indica A. Juss) on Aedes aegypti Linnaeus 1762 larvae. In African Journal of Biotechnology. 6 (24) : 2846 – 2845.
2. WHO. 2004. Panduan Lengkap Pencegahan dan Pengendalian DBD. Jakarta.
3. Nainggolan F. 2007. Epidemiology and Clinical Pathogenesis of Dengue in Indonesia. Seminar on Management of Dengue Outbreaks (22 Nov). Jakarta: UI.
4. Departemen Kesehatan RI. 2007. CDC and EH Yearly Report. Jakarta: Depkes RI.
5. Lei H.Y. 2007. Immunopathogenesis of the Dengue Virus caused disease.Presented at International Collaboration on Research.
6. Hua X, Biao D, Yu XP, Li WQ, Ya DW, Wei H, et.el. 2006. Serotype 1_Specific Monoclonal Antibody Based Antigen Capture Immunoassay for Detection of Circulating Nonstructural Protein NSI Implication for Early Diagnosis and Serotyping of Dengue Virus Infections; J. of Clinical Microbiology. p 2872-2878.
7. Sukowati S. 2010. Masalah Vektor Demam Berdarah Dengue (DENGUE FEVER) dan Pengendaliannya di Indonesia. Buletin Jendela Epidemiology, 2 : 27-29.
8. Widagdo L, Husodo, B, Bhinuri. 2008. Kepadatan Jentik aedes aegypty sebagai Indikator Keberhasilan Pemberantasan Sarang Nyamuk di Kelurahan Srondol Wetan, Semarang: Fakultas Kesehatan Masyarakat, Universitas Diponegoro.
9. Sungkar S, Rawina A. 2010. Pengaruh Penyuluhan Terhadap Tingkat Pengetahuan Masyarakat dan Kepadatan Aedes aegypti di Kecamatan Bayah, Provinsi Banten.Makara Kesehatan. 14 (2) : 81-85.
10. Kementrian Kesehatan RI. 2010. Demam Berdarah Dengue. Buletin Jendela Epidemiologi, 2 : 29.
11. Baskoro T, Nalim S. 2007. Pengendalian Nyamuk Penular Demam Berdarah Dengue di Indonesia. Disampaikan dalam Simposium Demam Berdarah Dengue. Yogyakarta : Universitas Gajah Mada.
12. Astari, S., dan Ahmad, I. 2005. Insecticide Resistance and Effect of Piperonyl Butoxide as a Synergist in Three Strains of Aedes Aegypti (Linn) (Diptera Cullicidae) on Insecticeide Permethrin, Sypermethrin, and d-Allethrin. Buletin Penelitian Kesehatan. 2 : 73 – 79.
13. Alexander L. 2002. How Does the Cabon Dioxide Mosquito Light Trap Work? http://www.articleinsider.com

14. Polson, K.A., Curtis, C., Seng, C.M., Olson, J.G., Chanta, M., Rawling, S.C.2002. The Use of Ovitrap Baited with Hay Infusion as a surveillance Tool for Aedes aegypti Mosquitoes in Cambodia. Dengue Bulletin.
15. Santos SRA, Melo-Santos MAV, Regis, L., and Albuquerque C.M.R. 2003. Field Evaluation of Ovitrap with grass infusion and Bacillus thuringiensis var israelensis to Determine Oviposition Rate of Aedes aegypti. Dengue Bulletin. 27: 156 – 162.
16. Geier M, Bosch OJ, Boeckh J. 1999. Ammonia as an Attractant Component of Host Odor for The Yellow Fever Mosquito Aedes aegypti. Chem Senses. 24: 647 – 653.
17. Kawada, H., Honda, S., Takagi. 2007. Comparative Laboratory Study on the Reaction ofAedes aegypti and Aedes albopictus to Different Attractive Cues in a Mosquito Trap.J. Med Entomol. 44(3):427-432.
18. Sterling, M.C., Lacey, R.E., Engler, C.R., Ricke, S.C. 2001.Effects of ammonia nitrogen on H2 and CH4 production during anaerobic digestion of dairy cattle manure. Bioresour. Technol. 77: 9 – 18.
19. Waluyo, L. 2005. Mikrobilogi Lingkungan. Malang: UMM Press.
20. Departemen Kesehatan RI. 2006. Tata Laksana Demam Berdarah Dengue. Jakarta: Dirjen PPM & PL Depkes RI.
21. Kasetyaningsih, T.W., Sri, S. 2006. Perbedaan antara House Indeks yang Melibatkan Pemerikisaan Sumur pada Survey Vektor Dengue di Dusun Pepe, Bantul, Yogyakarta. Jurnal Kedokteran Yarsi. 14(1): 034-037.
22. Sintorini, M.M. 2007. Peran Lingkunganpada Kasus Kejadian Luar Biasa Demam Berdarah Dengue. International Seminar on Mosquito-borne Disease Control through Ecological Approach. Yogyakarta.
23. Sudarmaja, I.M. 2008. Pengarus Air Sabun dan Detergen terhadap Daya Tetas Telus A. aegypti. Medicina. 39(1): 56 – 58.
24. Troyo, A., Calderon-Arguedas, O., Fuller D.O., Solano, M.E., Advendano, A., Arheart, K.L., Chade, D.D., Beier, J.C. 2008. Seasonal Profiles of Aedes aegypti (Dipthera Culicidae) Larva habitats in an urban area of Costa Rica with a History of Nosquito Control. J Vector Ecology: 33(1), 76 -88.
25. Wulandari TK. 2001. Vektor Demam Berdarah dan Penanggulangannya. Mutiara Medic. 1 (1): 27 – 30.
26. Phontas, A.S., Moer, M., Sucipto, H. 2012. Kepadatan Populasi LarvaAedes aegypti pada Musim Hujan di Kelurahan Patemon, Surabya. Skripsi. Departemen Biologi, Fakultas Sains dan Teknologi. Surabaya : Universitas Airlangga.
27. Jacquin, J. 2004. Insect Olfactory Receptors Contribution of Molecular Biology to Chemical Ecology. Diakses pada tanggal 25 November 2010.
28. Wiggleworth VB. 1972. The Principles Insect Physiology. London: Chapman&Hall
29. Muzaki A. 2007. Study Tentang Berbagai pH Air Kontainer Terhadap Kesukaan Aedes aegypti Untuk Bertelur di Kelurahan Kranji, Kecamatan Purwokerto Timur, Kabupaten Banyumas. Purwokerto: Jurusan Kesehatan Lingkungan, Poltekkes.
30. Hidayat, M.C., Santoso, L., Suwasono, H. Pengaruh pH Air Perindukan terhadap Pertumbuhan dan Perkembangan Aedes aegypti Pra Dewasa. Cermin Dunia Kedokteran. 119: 47-49.
31. Sudarmaja, I., Mardihusodo, S. 2009. Pemilihan Tempat Bertelur Nyamuk Aedes aegypti pada airAir Limbah Rumah Tangga di Laboratorium. Jurnal Veteriner, 10 (4) : 205-207.
32. Jumar. 2000. Entomologi Serangga. Jakarta : PT. Rineka Cipta.

THE EFFECT OF SOYBEAN (*GLYCINE MAX*) SOAKING WATER AS AN ATTRACTANT OF *Aedes sp*

Agustina Tri Endharti, Aswin D Baskoro, Nico Pangestu H
Faculty of Medicine, Brawijaya University, Malang, Indonesia
Correspondence: tinapermana@yahoo.com

ABSTRACT

Background: Attractant is one form of mosquito control by using media and materials that may attract mosquitoes. Materials would attract mosquitoes by producing CO_2 and ammonia. One of substances that are believed to have attractant potential is soybean soaking water. Soybean soaking water produces CO_2 and ammonia as attracting materials through mosquito's receptors. The purpose of this study was to determine the effect of soybean soaking water as mosquito attractant of *Aedes sp*.
Methods: This was an experimental study with post test control group design. There were five groups in the experiment, each consisted of 25 mosquitoes. Group 1 was provided with 50 g sugar and 1 g yeast soaking water in 200 ml tap water as a positive control. Group 2 provided with tap water only as a negative control Group 3-5 were provided with soybean soaking water with a dilution of ^{a} respectively.
Results: The data indicated that soaking water has attractant effect on *Aedes sp.* This study showed a significant difference on attractant potential between treatment and control group (ANOVA, $p<0.05$). Soybean soaking water in $^{3}/_{10}$ dilution had the greatest effect as attractants compare with other concentration.
Conclusions: It can be concluded that 3/10 dilution of soybean water soaking was the most effective attractant of *Aedes sp*.
Keywords: attractant, mosquito, soybean soaking water

BACKGROUND

Aedes sp mosquito has been identified to be one of the vectors that transmit the dengue virus from one host to another. The dengue virus will be in the blood of an infected host for about seven days. An uninfected mosquito that feeds on this individual during this period will acquire the virus. Once infected, the virus carried by the mosquito will be transmitted to another host during another blood meal. This transmission cycle will continue until there is no more infected mosquito [1]. Attractants and stimulants, especially, have great potential not only in detection and surveillance of mosquito populations and associated pathogenic viruses, but also in sustainable vector and disease suppression by targeting gravid mosquito females, which are epidemiologically the most important component of the mosquito populations [2]. Vector mosquitoes which have a major role in the spread of dengue infections are Aedes *aegypti* [3].

Dengue continues to increase in prevalence. Each year, there was an estimated 50-100 million cases of dengue fever and more than 500,000 cases of dengue hemorrhagic fever in the world. Indonesian data also showed that the incidence of dengue in

Indonesia is more than 50 cases per 100.000 populations with a mortality rate of around 1-2 percent [4]. One method of mosquitoes control is successful in reducing the density of *Aedes sp* in some countries by using attractant [5]

Gravid *Aedes sp* females prefer to land egg in organic infusions of fermenting leaves in water [6]. A variety of plant species and plant-associated materials have been used to produce organic infusions for investigating the oviposition behavior of mosquitoes or monitoring ovipositor activity in the field [2]. Other organic materials, such as sod and pelletized plant-based animal feeds, have been fermented to create infusions that were attractive to gravid *Aedes sp.* One of gravid females of mosquitoes is attractiveness of infusions. *Aedes sp* is attracted to hay and grass infusions [7].

Indonesia has produced many fermented soy beans. In this process, require a lot of water used for soaking, boiling, washing and stripping the skin of soybean. Waste obtained from the above process can be both liquid waste and solid waste. Liquid waste in the form of water soaking soybeans and soybean water discharged directly surrounding waters. If the waste is dumped directly in the river, it will cause a bad smile of H2S and ammonia as a result of the fermentation of organic waste. Ammonia and CO2 are mosquito attractants for the sensory receptors. Ammonia and carbon dioxide from Soybean can be used for attractants [8]. This study aimed to investigate the effect of soybean (*Glycine max*) soaking water with different concentrations as attractant of *Aedes sp*

METHOD

Design of experiment

This was an experimental research laboratory with post test only control group design.

Maintenance of mosquito colonies Aedes sp

Larvae 2nd stage of *Aedes sp* obtained from obtained from the Tropical Disease Center Airlangga University Surabaya. Larvae bred until become adult ± for 4-5 days in the Laboratory of Parasitology Faculty of Medicine, University of Brawijaya Malang.Ovitraps consisted of a black polyethylene cup 500ml,11 cm high, 6.5 cm wide at the bottom and 9 cm wide at the top, painted black on the outside, filled with water to within 2.5 cm of the top were placed in cage.

Preparation of soybean soaking water

Soybean soaking water was prepared by adding 0.5 kg of soybean to 10 liter well water, and then incubated for seven days at room temperature.The solution was added to the oviposition cup and well water added to make $^{1}/_{10}$, $^{2}/_{10}$ and $^{3}/_{10}$ dilutions.

Treatment

Weighing 0.5 kg soybean soaked in a bucket with 10 liters of water then left it for 1 week at room temperature. It will be made with the dilution, and then inserted into the ovitrap. There were 3 concentration of soybean soaked water such as $^{1}/_{10}$, $^{2}/_{10}$ and $^{3}/_{10}$.

There were 5 groups with different concentrations of soybean soaking water. One group was positive control (yeast 50 g and sugar 1 g in 200 ml of well water) and one other group was negative control (well water). Overall, the 5 groups were as follow: (1)

Ovitrap containing well water, (2) Ovitrap containing 50 g sugar + 1 g yeast, (3) Ovitrap containing $^{1}/_{10}$ dilution, (4) Ovitrap containing $^{2}/_{10}$ dilution, (5) Ovitrap containing $^{3}/_{10}$ dilution. Each ovitrap contains 500 ml of well water.

Ovitrap from cage that had filled with soybean soaking water in different concentrations was entered into a mosquito cage. The number of mosquitoes that landed on ovitrap will be counted and observed.Mosquitoes will be calculated in the same way on every hour until 6th hour. Repetition of the experiment will be conducted 4 times.To investigate the effect ofSoybean soaking water as an attractant on *Aedes sp*, ovitrap were placed in cage 40x40x40cm contain 25 mosquitoes.In experiment, each black cup was placed randomly in diagonal corners in cage. Cages were placed in room temperature (28±2°C; 70-90% RH).

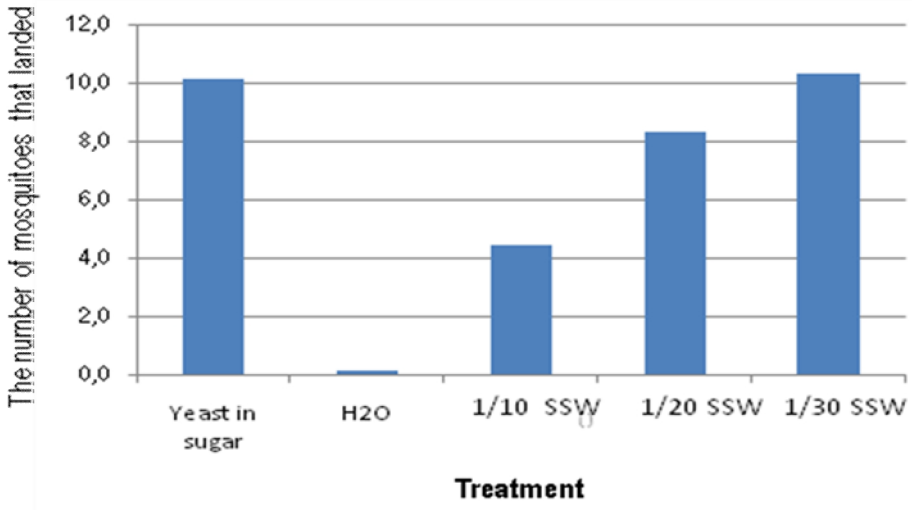
Statistical analysis

Results of calculation of the number of mosquitoes alight between control and treatment groups were statistically analyzed using SPSS 19 with a significance level of 0.05 (p = 0.05) and 95% confidence level (α = 0.05)

RESULTS

The effect of soybean soaking water as attractant

To enhance the attractiveness of ovitrap toward *Aedes sp,* we used different concentration of soybean soaking water. The results of Tukey HSD test found significantly differences in the number of mosquitoes had been trapped between positive control and negative control (p=0.000), between the positive control and soybean soaking water (p=0.000), positive control and soybean soaking water (p=0.003). While the analysis of the positive control (sugar and yeast medium) and soybean soaking water had similar between the treatments (0.990). In addition, no significant differences were found between treatment groups. Figure (1) indicates the number of mosquitoes that trap on ovitrap.



Note: SSW = Soybean Soaking Water

Figure 1. The average of mosquitoes landed on ovitrap with different concentrations

The ovitraps that contained soybean soaking water, there was increase the number of mosquitoes which trapped in ovitraps, there were containing well water (p =0.001) or $^{1}/_{10}$ $^{2}/_{10}$ soybean soaking water (p >0.001) or soybean soaking water (p >0.001) and $^{3}/_{10}$ soybean soaking water (p >0.001).

To investigate whether the attractiveness had effected with time point of mosquito's landed, we was observed by counting the number of mosquitoes had been trapped on ovitraps. The average number of mosquitoes on ovitrap was observed every hour until hour 6th. The number of mosquitoes were significantly increased in the ovitraps containing 3/10 soybean soaking water (p >0.001) compared to the control (Figure 2).

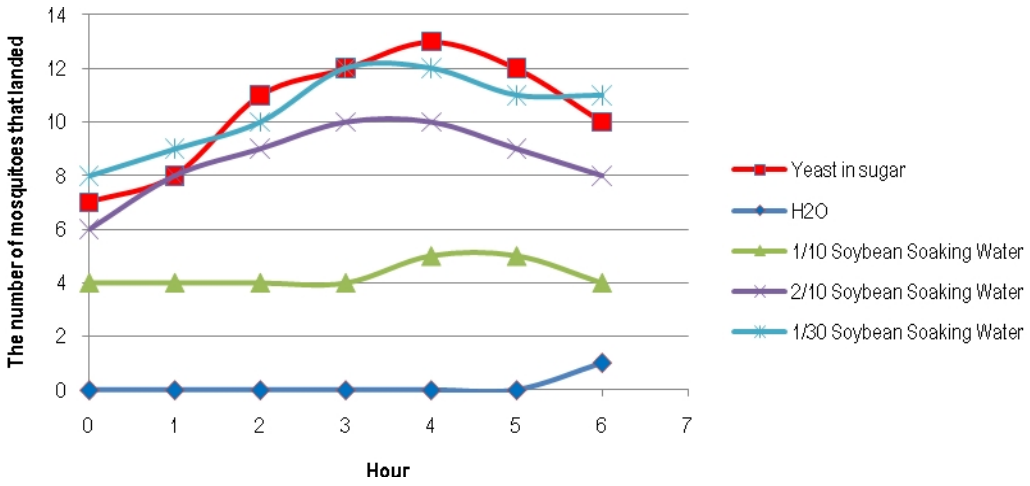


Figure 2. The average number of mosquitoes on ovitrap was observed every hour until hour 6th

DISCUSSION

This study used fermentation of soybean (*Glycine max*) soaking water because it contained ammonia and CO_2, which is one attractant of mosquitoes. This is supported by Carlos *et al (*2013) that the waste of soybean fermentation produce ammonia and CO_2. Ammonia and CO_2 are one of mosquito's attractant that has appeal for the sensory receptors of *Aedes sp*. The attractiveness was observed by counting the number of mosquitoes had been trapped on ovitraps [8].

In preliminary studies was found that a suitable time to soak soybeans for this study was 1 week. Based on Carlos *et al (*2013) showed soaking soybeans more than 1 week affect to behavior of mosquitoes. They were not interesting to come on ovitrap and loss sensitivity to response the attractant. This may occur due to increased ammonia levels are too high [8].

This study uses 3 different kinds of dilution. Dilution soybean soaking water was determined based on preliminary research. Positive control used was soaking water 50g sugar mixed with 1 g of yeast in 200ml of well water to produce CO_2. Ammonia and CO_2 cause a specific response to mosquitoes as attractants. The distinctive odor of

mosquitoes captured by the antenna, which consist of one or several nerve bipolar olfactory as ORNs (Olfactory Receptor Neurons). ORNs located at the end of the dendrites. The sensory nerves conduct electrical impulses to the chemical form of the response then it carries the information from the periphery to the olfactory lobe antenna which connects to the brain. Smells bind to OBPs (Odorant Binding Proteins) then passes through lymph fluid. OBPs are also working dissolve the odor molecules and act in the selection of olfactory information. When complex odor OBPs achieves dendrite membrane, the smell will binds to the trans-membrane receptor, and then it transferred to the intracellular membrane surface. Furthermore the electrical impulses delivered to brain and integrated to produce the response [9].

Figure 2 indicate that time point have effect on the maximum number of mosquitoes alight on ovitrap. This may be caused by increased levels of ammonia and CO_2 that is produced with increasing concentration as described in Santos *et al* (2003) study on the effect of the concentration of water soaking hay where there are increased levels of CO_2 and ammonia on any increase in the concentration of water soaked [5].

he result of Tukey HSD test found significant differences between the positive control and a negative control (p=0.000), between the positive control and soybean soaking water (p=0.000), positive control and soybean soaking water (p=0.003). The positive control with soybean soaking water is not found significant differences (0.990). This is due to the similarity between the results of the treatment. This similarity occurs because of the effect of Soy bean soaking water concentration $^{3}/_{10}$ had activity same as the positive control (yeast in sugar medium)

Soybean soaking water potential reduction might be due to the degradation of substances that contained the active substance molecules that form a complex odor-OBP at this dilution only slightly and the brain does not recognize it as an attractant. The more daylight time may also affect mosquito activity. In infusions made by fermenting some varieties of grass and hay species have been reported to be active towards gravid *Aedes aegypti* in laboratory and field bioassays [7].

At 6^{th} hour of experiment the sensory response of the mosquito may be decreases and less attracted to the attractant. From (figure 2) showed that at 5^{th} and 6^{th} hour, the number of mosquitoes decreases. It is due to the periodicity of *Aedes sp* sucking blood, especially during the morning hours 08.00-12.00 [9].

In this study also found that the relationship with the periodicity of the mosquito research time. According to Polson *et al* (2011) *Aedes sp* is diurnal and primarily active in the early morning hours till noon between 8:00 to 12:00 while research on everything from 7:00 to 13:00. The data indicated the number of mosquitoes that land start to decrease when entered at 12.00 then continued to decline in the next hour [9].

CONCLUSIONS

The increasing dilution water soaking hay then the number of mosquitoes that land is increasing. This experiment were conducted with concentrations of $^{1}/_{10}$, $^{2}/_{10}$ and $^{3}/_{10}$ dilution of soybean soaking water it which only the 3/10 dilution of concentration presented greatest attractive effect in *Aedes sp*.

REFERENCES

1. Yusoff N, Budin H, and Ismail S. 2012. Simulation of Population Dynamics of *Aedes* using Climate Dependent model. World Academy of Science, Engineering and Technology. Vol.62:477-482
2. Loganathan P, Ning X, Nojima S,Wesson WM, Coby S, and Charles S. 2008. Apperson Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by Aedes aegypti.PNAS vol. 105 :27: 9263
3. Loganathan P., Ning X.,Katalin B and Wesson DM. 2010. Oviposition Responses of the Mosquitoes Aedes aegypti and Aedes albopictus to Experimental Plant Infusions in Laboratory Bioassays. J Chem Ecol 36:709–719
4. Sutanto I, Ismid IS, Pudji K, Sjarifuddin, Sungkar S. 2008. Parasitologi Kedokteran. Edisi keempat.Departemen Parasitologi, FKUI, Jakarta.
5. Santos SR, Melo-Santos MA, Regis L and Albuquerque CMR. 2003. Field Evaluation of Ovitraps Consociated with Grass Infusion and *Bacillus thuringiensis* var. *israelensis* to determine Oviposition Rates of *Aedes aegypti.* Dengue Bulletin.Vol 27:15-18
6. Reiter P (2007) Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. *Vector-Borne Zoon Dis* 7:261–273
7. Sant'Ana AL, Roque RA, Eiras AE (2006) Characteristics of grass infusions as oviposition attractants to *Aedes* (*Stegomyia*) (Diptera: Culicidae). *J Med Entomol* 43:214–220
8. Carlos M. Baak B, Américo D. Ramírez R, Julián E. García R, Silvany RD, and Estrada JL. 2013.Development and laboratory evaluation of chemically-based baited ovitrap for the monitoring of *Aedes aegypti*.*Journal of Vector Ecology*38 (1): 175-181.
9. *Polson KA, Curtis C, Seng MC, Olson JG,Chantha N and Sam C Rawlins CS. 2002.*The Use of Ovitraps Baited with Hay Infusion as a Surveillance Tool for *Aedes aegypti* Mosquitoes in Cambodia*Dengue Bulletin* Vol 26:56-62.
10. Fachruddin. *2000.* Budidaya Kacang-kacangan. Kanisius. Yogyakarta. 20-30

BIOLARVACIDE ACTIVITY OF SOFTCORAL SYMBIONTS BACTERIAL *Sarcophyton* sp. SCRTG4P4 AGAINST *Aedes aegypti*

Sulistiyani, Retno Hestiningsih, Dian Nur Rahmawati
Public Health Faculty, Diponegoro University, Semarang
Correspondence: sulisbarkah@yahoo.co.id

ABSTRACT

Background: Biolarvacides is an alternative vector control for *Ae. aegypti*. Phytochemicals test from extract of bacterial symbionts *Sarcophytonsp*. SCRTG4P4 has Alkaloids, Terpenoids and Sesquiterpenoids there were indicated as larvacides function. The objective of the research was to determine the acitivity of biolarvacides of softcoral symbionts bacterial *Sarcophyton* sp. SCRTG4P4 against *Ae .aegypti*.
Method: This was an experimental study with post test only control group design. The variation concentrations of extract of bacterial symbionts softcoral SCRTG4P4 (0,1;0,20;0,30;0,40%), were applied to the III-IV instar of *Ae. aegypti* larvae. Data analysis carried out with one way ANOVA followed by Probit Analysis.
Results: The result showed there were significant differences of various concentration of extract softcoral *Sarcophyton* sp. SCRTG4P4 (p=0,000) and LC50 values of 0,275% and LC90 values of 0.40%.
Conclusion: Based on the results of ANOVA analysis, it was concluded various concentration of symbiont bacteri of softcoral have potency as biolarvacide.
Keywords: biolarvacide, *Ae.aegypti*, symbionts bacteria, softcoral

BACKGROUND

Bio-larvacides is an agent of insect control from biological agents that easy to apply. It is not harmful for natural enemies and other beneficial insects, and the one of eco-friendly to be an alternative insect control. Previous research showed that marine organism *Placospongia melobesiodes* had larvacides activity, and a tunicate *Ascidian* had larvacides activity against *Ae. aegypti.*

SCRTG4P4 is a symbiont bacterium from soft coral *Sarcophyton* sp.It has alkaloids, saponin and triterpenoids from phytochemical test result. Molecular identification from SCRTG4P4 with PCR test, Sequencing, and BLAST showed that SCRTG4P4 was *Bacillus subtilis*. *Bacillus subtilis* use as a fungicide fortunately does not affect humans. Some strains related to *Bacillus subtilis* are capable of producing toxins for insects. *Bacillus thuringiensis*, for example, is another bacterium in the same genus that is used for insect control.

The objective of the research was to determine the activity of biolarvacides of soft coral symbiont bacterial *Sarcophyton* sp. SCRTG4P4 against *Ae.aegypti*.

METHOD

Research was done by experimental method with post test only control group design. Bacterial symbiont soft coral *Sarcophyton* sp. SCRTG4P4 was come from the other research that have done before this research. Soft coral sample was taken from

Karimunjawa, Center of Java by Scuba Diving. Isolation of soft coral symbiont bacterial *Sarcophyton* sp. used streak method. Identification of molecular bacterial symbiont used PCR, Sequencing and BLAST. The variation concentrations of extract of bacterial symbiont soft coral SCRTG4P4 (0.15; 0.20; 0.25; 0.30; 0.35; 0.40; 0.45%) and control, were applied to the III-IV instars of *Ae.aegypti* larva. Data analysis carried out with one way ANOVA followed by Probit Analysis. Every treatment had 20 larva of *Ae.aegypti* and 3 repeated actions. The observation of larva mortality was every 2 hours for 24 hours.

RESULT

The study before found that molecular identification of SCRTG4P4 was *Bacillus subtilis* and from phytochemicals test showed it was contain alkaloids, triterpenoids and saponin. Bio-larvacide test showed the mortality of larva for each concentration of extract of bacterial symbiont soft coral Sarcophyton sp.SCRTG4P4 and control is in Table 1.

Table 1. The mortality of larva *Ae.aegypti*

Concentration (%)	Total Mortality	Mean of Mortality from 3 repeated	% of Total Mortality
Control	0	0	0
0.15	6	2	10
0.20	14	4.67	23.35
0.25	27	9	45
0.30	36	12	60
0.35	40	13.33	66.65
0.40	56	18.67	93.33
0.45	59	19.67	98.35

Normality data analysis with *Shapiro Wilk* showed normal distribution with p=0.642 ($p \geq 0.05$). The one way ANOVA with α=0.05 showed p=0.000. There were significant differences of various concentration extract of SCRTG4P4 as bio-larvacides to the mortality of larva *Ae.aegypti*. The Probit analysis showed bio-larvacides activity of soft coral symbiont bacterial *Sarcophyton* sp. SCRTG4P4 had LC_{50} value = 0.275% and LC_{90} value=0.40%.

DISCUSSION

SCRTG4P4 is symbiont bacterial marine organism especially from invertebrate soft coral *Sarcophyton* sp. Several marine organisms have adapted themselves through symbioticassociation among themselves that help them survive under harsh environments. There have been many examples of striking structural similarities between natural products obtained from marine invertebrates and those of microbial origin which suggests that microorganisms living in their invertebrate hosts could be the actual producers of these secondary metabolites. The limitation supply of marine animal and plant including marine invertebrates becomes the problem because of the low

content of active compounds; therefore, many researchers have focused on marine microorganisms as sustainable resources.

Molecular identification result showed that SCRTG4P4 is *Bacillus subtilis*. Bacillus is the gram-positive, aerobic, rod-shaped endospore-forming bacteria. Genus *Bacillus* arethe most widely represented organisms in the soil and aquatic environment. *B. cereus,B. subtilis*, and *B. pumilus*are considered as a major component of marine bacterial communities. *Bacillus subtilis* strains can act as biofungicides for benefiting agricultural crops and antibacterial agents. *Bacillus subtilis* use as a fungicide fortunately does not affect humans. Some strains related to *Bacillus subtilis* are capable of producing toxins for insects. *Bacillus thuringiensis*, for example, is another bacterium in the same genus that is used for insect control.

Based on the phytochemical test, bacterial symbionts SCRTG4P4 contained alkaloids, triterpenoids and saponin. Marine organism have been found to produce secondary metabolites such as alkaloid, terpenoid, peptides, sulfated polysaccharides, sesterterpeneand other new compounds. Alkaloids have function as toxin to insect as digestive toxin that can reduce the tense of tractus digestivus mucosal larva. The other way of alkaloids as insecticide is growth inhibition, especially to inhibit brain hormone, ediction hormoneand juvenile hormonethat make the failure of metamorphosis.

So it showed that SCRT4P4 had an activity as bio larvacides against *Ae.aegypti*. Bio larvacides is an agent of insect control from biological agents that easy to apply, is not harmful for natural enemiesand other beneficial insects, and the one of eco-friendly to be an alternative insect control.

CONCLUSION

There we significant differences of various concentrations extract as bio-larvacides activity of bacterial symbiont *Sarcophyton* sp. SCRTG4P4 against *Ae.aegypti* with significance p=0.000

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REFERENCES

1. Brooks GF, JS. Butel, and A. Morse. 2004. Jawetz, Melnick & Adelberg's Medical Microbiology, 23th. McGraw Hill Companies Inc. New York.
2. Madigan MT, M.Martinko J, F.Dunlap P, P.Clark D. Brock. 2009. Biology of Microorganism. 12th ed. Pearson Benjamin Cummings, San Francisco.
3. Khetan SK. 2001. Microbial Pest Control. Marcel Dekker Inc, New York.
4. Pratiwi ST. 2008. Microbiologi farmasi. Penerbit Erlangga, Jakarta.
5. Proksch P, RA. Edrada, R.Ebel., 2002. Drugs from the sea current status and microbiological implications. *Appl. Microbiol. Biotechnol*. 59:125-34.

6. Radjasa, OK, SIO. Salasia, A. Sabdono, J.Weise, JF. Imhoff, C. Lammler, and MJ. Risk. 2007b. Antibacterial activity of marine bacterium *Pseudomonas* sp. Associated with soft coral Sinularia polydactyla against Streptococcus equi subsp. Zooepidemicus. Int. J. Pharmacol. 3 (2):7-13
7. Radjasa OK. 2008. Bioprospeksi bakteri simbion invertebrata terumbu karang. Badan Penerbit Universitas Diponegoro, Semarang.
8. Sabdono A. 2008. Bacterial symbionts of reef's invertebrates: a marine natural drug's factory. J Coast Dev. 12(1):13-9.
9. Sulistiyani, Nugraheni SA, Khoeri MM, Sabdono A, Radjasa OK. 2010. Antibacterial activity of bacterial symbiont of softcoral *Sinularia* sp. against pathogenic resistant bacteria. J Coast Dev. 13(2):113-8.
10. Sulistiyani, Kusmita L, Radjasa OK, Sabdono A. 2011. Laporan Akhir Hibah Penelitian Strategis Nasional: Skrining Bakteri Simbion Karang lunak yang Memiliki Aktivitas Antibakteri terhadap MDR-TB. Universitas Diponegoro, Semarang.
11. Todar,K.2008.Bacterial structure in relationship to pathogenicity. http://textbookofbacteriology.net/BSRP.html. Access 16 July 2009.

ETHANOLIC EXTRACTS OF *BELUNTAS* LEAF (*Pluchea indica*Less.) AS LARVACIDE AGAINTS *Aedes aegypti* L. MOSQUITO

Suryadi Islami
Kelompok Studi Entomologi, Faculty of Biology UGM
e-mail: suryadi.islami@mail.ugm.ac.id

ABSTRACT

Background: Recent *Aedes* control mostly depends on chemical insecticide. The excessive use of chemical insecticides can cause resistance, environment pollution, and kill other targets. Bio-insecticides are friendlier to environment. *Beluntas* (*Pluchea indica*) is thought to have a bio-larvacide potency due to its alkaloid, flavonoid, tannin, atsiri oil, chlorogenic acid, natrium, calium, aluminium, calcium, magnesite and phosphor ingredients in its leaves. The purpose of this study was to investigate the influence and LC_{50} of ethanol extract of *beluntas* leaf against development of *Aedes aegypti* larvae.
Method: This study used completely randomized design (CRD) with one factor (concentration of ethanol extract of Beluntas leaf). The experiment used concentration of 0.8%, 1.6%, 2.4%, 3.2% and 4%. The control groups used water, DMSO, and abate 1%. Total volume used was 225ml. All groups tested 25 larvae. Each treatment repeated 3 times. Death of larvae was counted every 24 hours for 3 days. Analysis data used probit analysis, ANOVA and then continued with Duncan's Multiple Range Test (DMRT) test.
Results: The result showed an average mortality of larvae varied. The higher the concentration the higher the mortality. The average mortality of larvae showed in chronological order from the smallest to the largest concentration was 13.33, 33.33, 40, 45.33 and 58.67% respectively. The result showed that ethanol extract of *beluntas* leaf significantly caused the larvae death ($p < 0.001$). The concentration to effectively kill 50% larvae (LC_{50}) was 3.325%.
Conclusion: Based on results, it was concluded that the ethanolic extracts of *beluntas* leaf (*Pluchea indica* Less.) were potential to be larvicides of *Aedes aegypti* larvae.
Keywords: larvacide, *Pluchea indica* Less., *Aedes aegypti* L., LC_{50}.

INTRODUCTION

Indonesia is a country with cases of Dengue Fever every year. According WHO (2009), Indonesia was the country with the highest incidence of dengue in Southeast Asia from 1968 to 2009 [1]. Dengue was a disease caused by the dengue virus was transmitted through vectors *Aedes mosquito*. Dengue virus spread by *Aedes mosquitoes* has been extremely fast and can cause death in a short time. This is an event that must be dealt with on target and fast to avoid the occurrence of outbreaks [2].

Treatment for this case commonly is spraying insecticide to kill adult mosquitoes. Currently, control uses chemical insecticides [3]. However, the use of chemical insecticides can cause resistance, environmental pollution, and noton the target [4].

Therefore, proper control was to usefriendlyenvironmental biological insecticides from chemical plant [5].

Indonesia is a mega-biodiversity country of flora [6], which one of it was beluntas (*Pluchea indica*). Beluntas was currently more widely used as a spice in cooking and hedgerow [7], but beluntas had the potential to be used as a herbal insecticide[8]. According to Riyanto[7], beluntas can be used as a herbalinsecticide on green bean beetle (*Callosbruschus chinensis* L.) (Coleoptera: Bruchidae). Chemical constituents in the beluntas leaves were also varities. According to Susetyarini [8], the beluntas leaves contain alkaloids, flavonoids, tannins, essential oils, chlorogenic acid, sodium, potassium, aluminum, calcium, magnesium and phosphorus. This study aimed to determine the effect of ethanol extract and LC_{50} values of beluntas leaves on the development of *Aedes aegypti* larvae.

METHOD

Beluntas leaves were extracted using 96% ethanol and aquades in the Laboratory of Pharmacy UGM. The extract was diluted with DMSO. Mosquito larvae obtained from rearing in Laboratorium of Parasitology UGM. *Aedes aegypti*was breed in container containing wells water.Pellets was used to feed the larvae, marmotto used to feed adult female mosquitoes, andsugar water used to feed adult malemosquitoes. Preliminary test was conducted to obtain the percentage of extract at the actual test. Concentrations used were0.1%, 0.2%, 0.4%, 0.8% and 1.6%, and control were water, water mixed with DMSO, and water mixed abate1%. Larvae to be tested as many as 25for each concentration with 3 replications. The water volumethat used was 225 ml. The mortality of larvae was calculated every 24 hours for the concentration range obtained for 3 days. Preliminary test results obtained were used as a basis for determining the concentration of the treatment on the actual test. The method of actual test was same as the preliminary test. Development and larvae mortality were analyzed using Probit analysis to determine LC_{50}. To determine the significance results between the treatment with the control done by ANOVA test and DMRTtest

RESULTS

Table 1. Total and mortality of *Aedes aegypti* larvae for 72 hours on the preliminary test

Treatment	Total of larvae tested	The total number of larvae died						The averages of larvae died ± SD	%
		I		II		III			
		Larvae died	%	Larvae died	%	Larvae died	%		
Water control	25	0	0	0	0	0	0	0 ± 0	0
DMSO control	25	0	0	0	0	0	0	0 ± 0	0
abate 1 % control	25	25	100	25	100	25	100	75 ± 0	100
0.10%	25	1	4	1	4	0	0	2 ± 0.58	2.66
0.20%	25	0	0	0	0	3	12	3 ± 1.73	4
0.40%	25	4	16	0	0	3	12	7 ± 2.08	9.33
0.80%	25	5	20	1	4	3	12	9 ± 2	12
1.60%	25	12	48	3	12	11	44	26 ± 4.93	34.66

The preliminary test used levels of concentration of 0.1%, 0.2%, 0.4%, 0.8% and 1.6%. The mortality of preliminary test results varied by concentrations, but the higher concentration, the percent of mortality was also higher (Table 1). Preliminary test results showed no levels of concentration that kills 50% of the *Aedes aegypti* larvae population each treatments and each replications, the highest concentration (1.6%) can only kill an average 34.66 % of the population, so that from the results can be seen that it had not obtained LC_{50} values. In the actual test, because the preliminary test results had not been able to kill 50% of the larvae population so the level of concentration that was used must be above 1.6 %. Concentrations used in the actual test was 0.8 %, 1.6 %, 2.4 %, 3.2 %, and 4 %, in addition to the concentrations used were also three controls, were watercontrol, DMSO control and abate control 1 %.

Actual test results showed that mortality also varies, the higher the concentration the higher the percentage of mortality. On the control of water and DMSO control absence of larvae died there (0%), so the larvae diedin the control was <20%, which meant the actual test was acceptable and did not need to be repeated. Absence of DMSO control larvae that died demonstrated that DMSO was not toxic to the *Aedes aegypti*larvae, so it did not affect to the concentration and ethanol extracts of the beluntas leaves although DMSO was used here as a diluting solution.

Table 2. Total and mortality of *Aedes aegypti* larvae for 72 hours on the actual test

Treatment	Total of larvae tested	The total number of larvae died						The averages of larvae died ± SD	%
		I		II		III			
		Larvae died	%	Larvae died	%	Larvae died	%		
Water control	25	0	0	0	0	0	0	0 ± 0	0
DMSO control	25	0	0	0	0	0	0	0 ± 0	0
abate 1 % control	25	25	100	25	100	25	100	25 ± 0	100
0.80%	25	4	16	4	16	2	8	3,3 ± 1,15	13,33
1.60%	25	10	40	7	28	8	32	8,3 ± 1,53	33,33
2.40%	25	10	40	9	36	11	44	10 ± 1	40
3.20%	25	11	44	12	48	11	44	11,33 ± 0,58	45,33
4%	25	14	56	14	56	16	64	14,67 ± 1,15	58,67

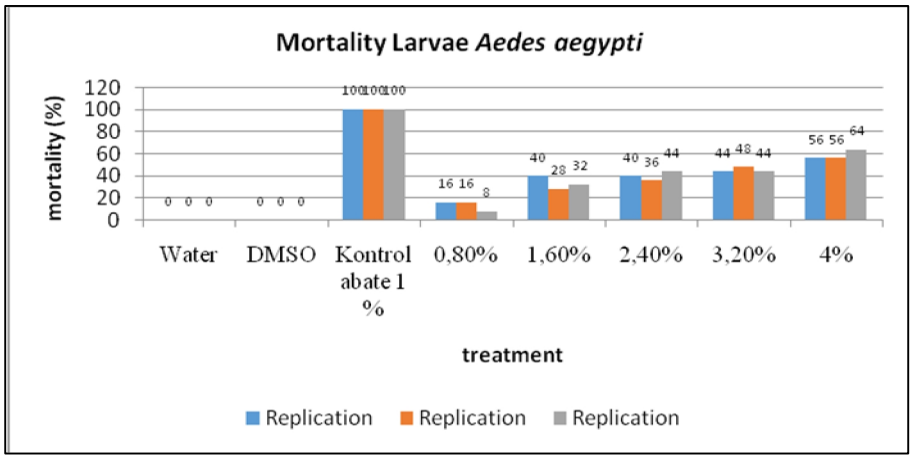

Figure 1. *Aedes aegypti* larvae mortality during the 72 hourstreatment

The actual test results showed that there was existing concentration that killedthe larvae population 50%, the concentration range was between concentration 3.2% (average of larvae mortality 11.33) and the concentration 4% (average of larvae mortality 14.67). Based on continuation the results of probit analysis was performed to determine its LC_{50} value. The data used was the average mortality of larvae for 72 hours, with a value of y = 12.5 which was 50% population of larvaes (the number of larvaes testing was 25 larvae). Probit analysis results can be seen in Figure 2.

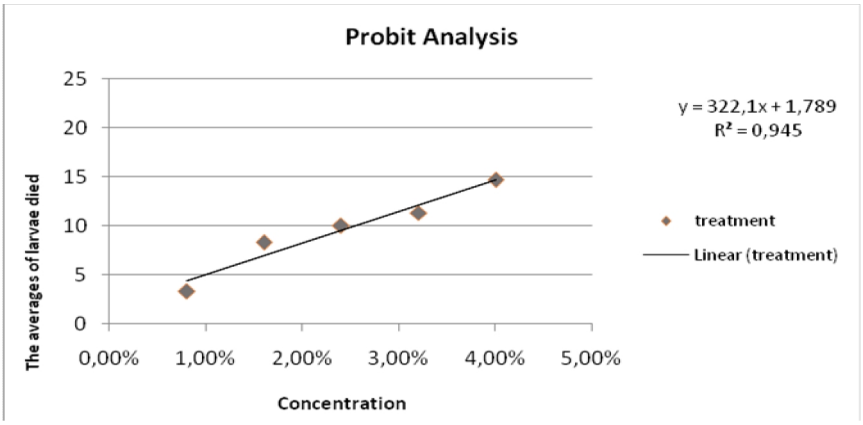

Figure 2. Result of LC_{50} value using probit analysis

Probit analysis results shows the equation y = 322.13 x + 1.789 with a regression value of R = 0.9726, from the equation obtained x value was the concentration that killedright larvae as much as 50% (LC_{50}) of population (25 larvae), the amount concentration was 3.325 %. Furthermore, the actual test results conducted analysis of variance (ANOVA) using software IBM SPSS Statistics 20. ANOVA results showed that there was significant difference ($p<0,05$). The results can be seen in Table 3.

Table 3. ANOVA Analysis

Tests of Between-Subjects Effects

Dependent Variable: *The numberof larvae died*

Source	*Type III Sum of Squares*	df	*Mean Square*	F	Sig.
Corrected Model	1467,167[a]	7	209,595	264,752	,000
Intercept	1980,167	1	1980,167	2501,263	,000
L	1467,167	7	209,595	264,752	,000
Error	12,667	16	,792		
Total	3460,000	24			
Corrected Total	1479,833	23			

a. *R Squared* = ,991 (*Adjusted R Squared* = ,988)

The ANOVA results showed a highly significant difference that needed to be done DMRT analysis with the same software, DMRT test results can be seen in Table 4.

Table 4. DMRT Analysis

The total number of larvae died								
Treatment		N	*Subset*					
			1	2	3	4	5	6
Duncan[a,b]	1	3	.00					
	2	3	.00					
	4	3		3.33				
	5	3			8.33			
	6	3				10.00		
	7	3				11.33		
	8	3					14.67	
	3	3						25.00
	Sig.		1.000	1.000	1.000	.085	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = ,792.
a. *Uses Harmonic Mean Sample Size* = 3,000.
b. *Alpha* = 0,05.

Based on DMRT analysis results, it shown that each treatment had different result but on treatment 1,6% and 2,4% showed that no different result.

Environmental parameters measured were temperature and pH, the parameter was measured every 24 hours for 72 hours (Table 5). Based on the results of these measurements, the temperature of each treatment and each replication did not change, that was, from the beginning measurement until the end of measurement at the temperature 27^0C. This showed that there was no difference temperature between the control treatments with concentrations so that treatment can be assumed that the temperature did not affect the value of larvae mortality testing. The results of pH measurement each treatment and each replication showed a decrease in pH at high concentrations, such as at the concentration of 2.4%, 3.2% and 4%, but the decline was not significant because it was only decrease 0.01 compared to the concentrations below and compared with controls. The decrease was possible because the higher concentration of the solution was so little acidic compared to the others, but with pH 6.9 can still be said to be neutral.

DISCUSSION

Based on the observations and data analysis, ethanolic extract of beluntas leaves can kill *Aedes aegypti* larvae. This case can happen due to a variety of content beluntas leaves which were toxic to the larvae of *Aedes aegypti*. According to Atmajaya [9], beluntas leaves extract contains tannins, flavonoids and alkaloids, the levels of the content was high enough, the content of tannins in extracts beluntas leaves at 2.351 %, 4.158 % of flavonoids and alkaloids amounted to 0.316 % [10].Flavonoids were compounds that can serve as an antimicrobial that can be toxic, these compounds synthesized by plants to protect themselves from pathogens [11] and tannin to alkaloid

can also be toxic to insects [12]. According to Cahyadi [13] flavoniods and alkaloids were toxic tend poisoning stomach (stomach poison). This compound will enter into the insect body through the digestive tract, which will be circulated through the blood circulation, affect the nerve, so that it will inhibit sense receptors in the mouth[10]. Larvae will fail to get a sense stimuli that can not be recognized the food and ultimately died [14].

Based on the observation that dead larvae by using a microscope, it can be seen that there was no body part that was damaged. It can be assumed that the ethanolic extract of beluntas leaves did not damage the structure of the larvae body and go through the digestive tract, so that the extract can be categorized as a stomach poison. Beluntas leavesethanolic extract can also be categorized as a neurotoxin that interfered with nerve on the mouth organ so that it can not be recognized its food.

CONCLUSION

Based on results, it was concluded that the ethanolic extracts of beluntas leaf (*Pluchea indica* Less.) were potential to be larvicides of *Aedes aegypti* larvae. It can kill the larvae of *Aedes aegypti*because itwas poison in the stomach and nerve of the larvae. LC_{50} value of Beluntas leaves ethanolic extract to*Aedes aegypti*was 3.308 %.

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REFERENCES

1. WHO. 2009. Panduan Lengkap Pencegahan dan Pengendalian Dengue dan Demam Berdarah Dengue. Jakarta. EGC.
2. Departemen Kesehatan RI. 2005. Tata Laksana DBD. Jakarta. Departemen Kesehatan RI.
3. Tarumingkeng, R. C. 1992. Insektisida; Sifat, Mekanisme Kerja dan Dampak Penggunaannya. UKRIDA Press.
4. Hascoet, M. 1988. Detection of Pesticide Residues. New York. Lavoisier Publ. Inc.
5. Ndione R. D., Faye O., Ndiaye M., Dieye A.& Afoutou J.M. 2007. Toxic effects of neem products (Azadirachta indica A, Juss) on Aedes aegypti Linnaeus 1762 larvae.African Journal of Biotechnology 6 (24)
6. Sukara, E. & I. SL. Tobing. 2008. Industri Berbasis Keanekaragaman Hayati, Masa Depan Indonesia. Vis Vitalis 2 (1).
7. Riyanto. 2009. Potensi Lengkuas (Languas galanga L.), Beluntas (Pluchea indica L.), dan Sirsak (Annona muricata L.) sebagai Insektisida Nabati Kumbang Kacang Hijau Callosobruchus chinensis L. (Coleoptera : Bruchidae). Saintmatika. 6 (2).
8. Susetyarini, E. 2007. Pengaruh Dekok Daun Beluntas terhadap Ld 50 -Toksisitas Akut Tikus Putih Jantan (Ratus norwegicus). Laporan Penelitian Pengembangan Ipteks. Lembaga Penelitian Universitas Muhammadiyah, Malang.

9. Atmajaya, F. D. 2013. Deteksi Senyawa Alkaloid, Flavonoid, dan Tanin pada Daun dan Batang Beluntas (Pluchea indicaLess.) dengan Metode Kromatografi Lapis Tipis. Laporan Seminar. Fakultas Biologi Universitas Gadjah Mada. Yogyakarta.
10. Sulistiyaningsih. 2009. Potensi Daun Beluntas (Pluchea indica Less.) sebagai Inhibitor terhadap Pseudomonas aeruginosa Multi Resistant dan Methicillin resistant Stapylococcus aureus. Penelitian Mandiri Universitas Padjadjaran Bandung. Hlm.18-22.
11. Hollingworth, R.M. 2001. Inhibitors and Uncouplers of Mitochondrial Oxydative Phosphorylation dalam Krieger R., J. Doull, D. Ecobichon, D. Gammon, E. Hoyson, L. Reiter & J. Ross. Handbook of Pesticide Toxycology Vol. 2. Academic Press. San Diego.
12. Nursal & E. S. Siregar. 2005. Kandungan Senyawa Kimia Ekstrak Daun Lengkuas (Lactuca indica L,), Toksisitas dan Pengaruh Sub Letalnya terhadap Mortalitas Larva Nyamuk Aedes aegypti L. Laporan Hasil Penelitian Dosen Muda FMIPA Universitas Sumatera Utara. Medan.
13. Cahyadi, R. 2009. Uji Toksisitas Akut Ekstrak Etanol Buah Pare (Momordica charantia L.) Terhadap Larva Artemia salina Leach Dengan Metode Brine Shrimp Lethality Test (BST). Skripsi. Semarang: Universitas Diponegoro.
14. Prabowo, H. 2010. Pengaruh Ekstrak Daun Nerium oleander L. Terhadap Mortalitas dan Perkembangan Hama Spodoptera litura Fab. Biota15 (3).

TRANSMISSION OF GERMAN COCROACH (*Blatella germanica*) EXPOSED BY *Metarhizium anisopliae* AND *Beuaveria bassiana*

Yayan Sanjaya, Saefudin, Kusnadi, Suhara
Indonesia University of Education
Correspondence: yayansanjaya229@gmail.com

ABSTRACT

Background: Cockroach plays an important role as a vector of various diseases. One way to control cockroach is the use of entomopathogenic fungi i.e. *Metarhizium anisopliae* and *Beauveria bassiana*. The application can be considered effective to kill cockroach by dispersing conidia. The aim of this study was to investigate transmission phenomenon from infected female to male by mating process and from cockroach female to its progeny.
Method: This is an experiment study with randomized design. There were two activitis 1) effect of infected female to male of cocroaches with three treatments by 107, 10^6 and 10^5 conidia/ml with 5 replications. 2) sub lethal effect of female cocroaches infected with 10^5 and 10^4 conia/ml with 5 replications
Results: The result showed the effect of auto-dissemination of *Metarhizium anisopliae* and *Beauveria bassiana* from infected female to male cockroach by mating process from female's genital. It was also found that higher concentration related to mortality. While in lower concentration, mortality was due to disruption on forming ootheca and producing its progeny.
Conclusion: It is indicated that entomopathogenic fungihas a potential effect on cockroach control.
Keywords: *Blatella germanica*, *Metharhizium anisopliae*, *Beauveria bassiana*, auto-dissemination, mortality

BACKGROUND

Development of science and technology today has helped to overcome the environmental problems of controlling cockroach [1]. One of the control method is using biological agents, such as fungi and bacteria that are pathogenic, and other insects that can control *Blatella germanica* cockroaches [2]. According to McCoy [3] a biological control program using natural enemies and pathogens organisms to environmentally friendly pest control number. The effort is expected to be stabilizing agriculture and safe for human life.

Currently, Integrated Vector Control attempted to use entomopathogen fungi [5]. Entomopathogen fungi that commonly used are *Beauveria bassiana, Verticillium lecanii* and *Metarhizium anisopliae* [6]. *M. anisopliae* can infect several insects of the Order Coleoptera, Lepidoptera, Hemiptera and Isoptera [7]. Spreading of the fungi can be horizontal or vertical. To reduce the population in the houses, *M. anisopliae* and *B. bassia*na were infected to female cockroach, which will influence on male cockroach

cockroaches when mating. This phenomenon may reduce the population of cockroaches [8].

METHOD

This research was conducted in FPMIPA Biology Education University of Indonesia with auto-dissemination test by Complete Random Design (RAL) with 4 treatments and 5 replications. Treatment is the concentration of entomopathogen spores 10^7, $10^6$and 10^5.

Rearing of German Cocroach (Blatella germanica)

Blatella germanica was collected from hotels, restaurants, trains and buses. Insects rearing were done in Ecology laboratory-UPI on condition of 26 $^oC \pm 2^o$ C temperature, 70-80% relative humidity and dark-light periods of 12: 12. Adult *B. germanica* consisting of 2 pairs of males and females were placed in plastic containers, then fed and given water wafers. For shelter and were given pieces of paper to the cockroach's eggs are placed in tissue paper (Figure 1).

Cultivation of pure fungal isolates entomopathogen

To create a pure isolates of the fungus or reproduce entomopathogen, *Metarrhizium anisopliae* were taken from the German cockroach insects that attack each fungi. The method was as follows: (a). All materials/substances making up this medium were weighed and measured according to rule; (b). Potatoes that have been washed clean were cut small dice-shaped size 1x1 cm, inserted into a 500 ml aquadest that have been heated to boil and continued for 1 hour (until the potatoes are soft and out of extract), (c). Meanwhile, stem agar was put in 300 ml aquadest and simmered until melted; (d). During that time the volume of potato extract and agar is maintained; (e). Next, sterile water was added to extract of the remaining potatoes and agar while still heated to boiling; (f). Potato extract liquid obtained in agar then filtered separately in hot conditions; (g). After that, extract the filtrate into dextrose included potatoes and yeast extract, stirring until dissolved homogeneous then inserted to dilute the results of screening; (h). Mixture of potato extract and agar if the volume is less than 1 liter plus aquadest up to 1 liter volume; (i). The mixture was then simmered, stirring frequently; (j). After all the soluble material, at the time of the fire is almost boiling off, the solution put into tubes and sterilized in the autoclave (121 ^{0}C temperature, pressure of 15 PSI for 30 minutes).

Auto-dissemination Test

Female German cockroach was dipped into petri dish containing *M. anisopliae* spore suspension by over 60 seconds. Infected female cockroaches were then placed in pairs of male guinea pigs. This procedure replicated 5 times. The concentrations used were 10^7, 10^6 and 10^5 and 10 visits resulting in the death of cockroach.

Analysis

Data were analyzed by Anova followed by Duncan's multiple range test.

RESULT

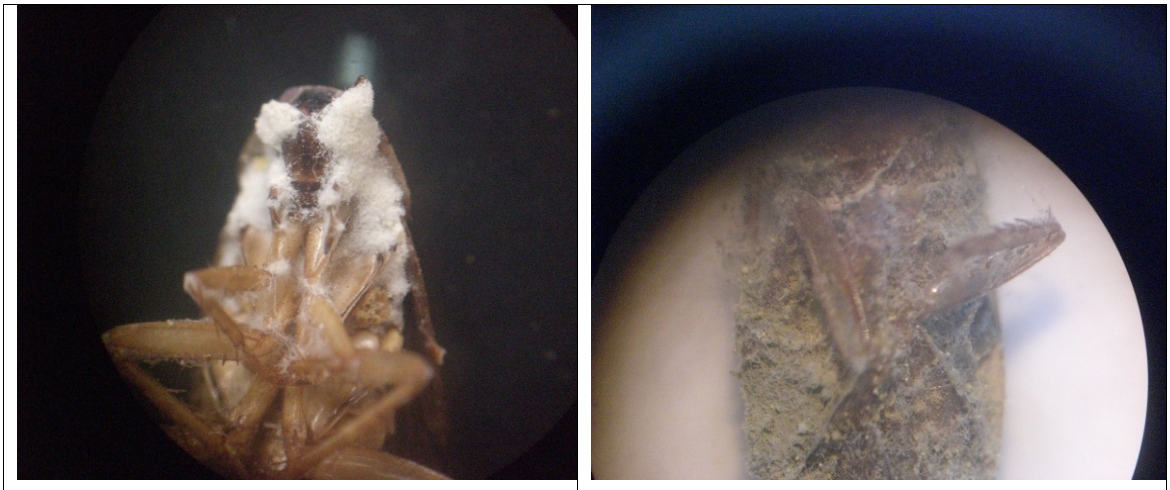
Observational data of horizontal effects of *Metarhizium anisopliae* and *Beauveria bassiana* that infected *B. germanica* can be seen on Table 1.

Table 1. Horizonal effects of *Metarhizium anisopliae* and *Beauveria bassiana* against uninfected *Blatella germanica*

	Metarhizium anisopliae	*Beauveria bassiana*
10^5	0 a (A)	0 a (A)
10^6	60 ± 2.5 b (B)	30 ± 3 b (A)
10^7	100 c (B)	60 ± 5 c (A)
Kontrol	0 a (A)	0 a (A)

Note: The figures in the column and (lines) are marked with the same same letter means do not differ according to Duncan's multiple range test on the real level of 5%.

It can be seen that both the treatments of *M. anisopliae* and *B. bassiana* could affect male cockroach death. For each treatment either both showed higher concentration gave higher in mortality respectively. Mortality of treatment of *M.anisopliae* on the 10^7 spore/ml was 100% which is the highest mortality of cocroach. While cocroach mortality on the same concentration on. *B. bassiana* caused 60% of mortality. On the other hand, treatment of both fungi on 10^7 was give no effect. Furthermore, in this case *Metarhizium anisopliae* was powerful than *B. bassiana*.



Beauveria bassiana | *Metarhizium anisopliae*

Figure 1. Horizonal effects *Metarhizium anisopliae* and *Beauveria bassiana* Against *Blatella germanica*

Figure 1 showed the mycelia spreading on *B. bassiana* and *M.anisopliae*, both of figure we displayed of spore on the abdomen of male cocroach, after each male sexually mating with infected female by copulation. The spore transmittes to male reproductive organ by attached, penetrated of cuticle and hypa formation.

DISCUSSION

The effect of female cockroach infection to the horizontal death by of male cockroaches, as cockroaches are going to do male copulation with a female cockroach. Cockroaches previously healthy male will sick even death due to female infected by the male cockroach is to penetrate. Cockroaches females are the first infected and only male cockroaches infected fungi, this is one of the phenomenon insect fungus can infect horizontally. This is as well as Scholte [5] study which examined the infected female mosquito then paired with healthy males and healthy male mosquitoes will be infected with *M. anisopliae* and *B. bassiana* due process of copulation.

Fungal dissemination within a host population can be occurs within activities and movements of the host [9]. The fungus can exploit host behavior like gathering, communicating, grooming (in social insects) and mating to spread through a host population [10]. The physiological state of the females and the natural display of behavior at the time of the bioassays, it is assumed that the observed auto-dissemination of *M. anisopliae and B. bassiana* from female to male *B. germanica* were the result of mating [11]. This is deeply supported by the findings from experiment where none of the males that had stayed on the surface area where fungus-contaminated females had rested previously acquired an infection.

The average age at death of fungus-infected mosquitoes was quite high when compared to mosquito survival in Scholte *et al* [5]. This is probably due to the relatively low level (10^5) of innoculum transferred. From those cockroach that were checked under the microscope for the presence of conidia, four out of one male contained conidia. It is thus likely that many males become contaminated, but that only a relatively low proportion of these males will actually succumb to the infection: in many cases the number of conidia was low, resulting in marginal infections that were successfully countered by the immune responses staged by the males.

In order to achieve the highest possible impact of the fungus on the mosquitoes population, it is desirable that other pathways besides the primary mode of (direct) contamination are utilized. The results of this study show that under laboratory conditions horizontal transmission can occur, which suggests that it may occur in the field. When these experiments were carried out, it was presumed that predominantly females would be infected directly from the indoor resting targets.

CONCLUSION

Based on the data and the picture above can be seen that *M. anisopliae* and *B. bassiana* can be spread with the spread of auto-dissemination females to males. This could be a reference for controlling cockroaches in homes

REFERENCES

1. Lyon WF. German Cockroach [Online]. Available at: http://ohioline.osu.edu/hyg-fact/2000/2099.html.
2. Shi WB, Feng MG. Lethal Effect of *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces fumosoroseus* on the Eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with A Description of A Mite Egg Bioassay System. Biological Control 2004;30, 165-173

3. McCoy C, Quintela ED, de Faria M. Environmental Persistance of Entomopathogenic Fungi [Online]; 2005.
Available: http://www.agctr.lsu.edu/s265/mccoy.htm.
4. Scholte EJ, Bart GJ Knols, Takken W. Auto-dissemination of the entomopathogenic fungus *Metarhizium anisopliae* amongst adults of the malaria vector *Anopheles gambiae s.s.* Malar J. 2004; 3: 45.
5. Barker CW, Barker GM. Generalist Entomopathogens As Biological Indicators of Deforestation and Agricultural Land Use Impacts on Waikato Soils. New Zealand Journal of Ecology. 1998; 22(2): 189-196. New Zealand Ecological Society.
6. Boucias DG, Pendland JC. 1998. Principles of Insect Pathology. New York:Academic Press.
7. AhmedS, Ashraf MR, Hussain A. and Riaz MA. 2009. Pathogenicity of isolates of Metarhizium anisopliae from Gujranwala (Pakistan) against Coptotermes heimi (Wasmann) (Isoptera: Rhinotermitidae). Int. J. Agric. Biol.11(6): 2009
8. Miller DM and Koehler PG. (1993). Least Toxic Methods of Cockroach Control. Available: http://edis.ifas.ufl.edu/scripts/htmlgen.exe?DOCUMENT_IG115.
9. Barnet, H.L. dan B.B. Hunter. (1972). Illustrated Genera of Imperpect Fungi. Fourt edition. Macmillan Publishing Company, New York. Collien Macmillan Publishers, London.
10. Burges, H.D. (1970), Microbial Control of Pests and Plant Diseases, 1970-1980. Academic Press, London.
11. Zurek L., D. W. Watson, and C. Schal. 2002. Synergism between Metarhizium anisopliae (Deuteromycota: Hyphomycetes) and boric acid against the German cockroach (Dictyoptera: Blattellidae). Biological Control 23: 296-302.

ENVIRONMENTAL MANAGEMENT OF LYMPHATIC FILARIASIS VECTOR

Sri Malem Indirawati
Sekolah Tinggi Ilmu Kesehatan Sumatera Utara
Correspondence: srimalem_indirawati@yahoo.co.id

ABSTRACT

Background: Filariasis is endemic in Indonesia and has become a public health problem. The number of reported cases of filariasis continues to grow. Filariasis is an infectious disease caused by filarial worms; which is transmitted by various species of mosquitoes. Therefore, the control of filariasis needs vector management.
Results: Vector management for prevention of filariasis can be done through the identification of the type of mosquito species by breeding places. Environmental management efforts in the control of filariasis vector control can be done through physical environments, biology and chemistry. The ongoing filariasis elimination program in Indonesia determined to achieve the elimination of two pillars. They are: 1) to break the chain of infection by mass drug administration in endemic areas, and 2) To prevent and limit disability due to filariasis.
Conclusion: Environmental management can be done by disconnecting life cycle vector. Efforts are the modification and manipulation of the environment.
Keywords: filariasis, vector management

INTRODUCTION

Filariasis is a disease caused by infection with the filarial worms that are transmitted by mosquitoes [1]. The disease is widely spread in rural and urban areas, and attacks all groups regardless of age and gender [2]. In the world there are 1.3 billion people at risk of contracting the disease in more than 83 countries and 60 % of cases are in Southeast Asia [3]. The disease can lead to disability, stigma, psychosocial and decreased productivity of patients and their environment [4]. Filariasis spread in almost all parts of Indonesia. From year to year the number of reported cases of filariasis in the province continues to grow. Even in some areas have a fairly high level of endemicity. Data on 2009 showed the number of patients continue to experience an increase in cases of filariasis be 11 941 people in 495 districts/cities in Indonesia [5].

Since 1999 the WHO to prioritize disease elephantiasis to be eliminated. In 2000 strengthened the WHO decision to declare "The Global Goal of Elimination of Lymphatic Filariasis as a Public Health Problem by the Year 2020". Indonesia agreed to eliminate filariasis as part of the global filariasis elimination to implement filariasis elimination programs since the year 2002 until 2020 [6].

Efforts have been done to control filariasis, yet because of existing constraints the resulting outcome is not maximized [7]. Mass treatment and prevention efforts have been made but there are still people with filariasis in Indonesia today. Under the government program since 2002 to date elimination effort has run for 11 years, 7 years remaining problem solving filariasis elimination in Indonesia [6]. Environmental

management is an effort on filariasis vector management, where the goal is to break the chain of transmission of vector [7]. This paper will explain further the efforts of environmental management that aims to break the life cycle of filariasis vector.

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RESULTS

Filariasis can be found in rural, coastal, and urban[2]. This transmission is distinguishedby the environment and species of mosquitoes. Indonesia is the only country in the world with three different types of worms that cause filariasis, namely *Brugia malayi*, *Brugia timori*, and *Wuchereria bancrofti*. Of the three types of worms, *Brugia sp* has the most wide spread in Indonesia, usually endemic in the rice field areas. These worms can be transmitted to a healthy person through the bite of mosquitoes like *Anopheles sp, Mansonia sp*, and *Culex sp* [8].

Filariasis endemic areas are generally low-lying areas, especially in rural, coastal, inland, rice fields, marshes and forests. In general, filariasis *W. bancrofti* spreads in Sumatra, Java, Kalimantan, Sulawesi, Nusa Tenggara, Maluku and Papua. *W. bancrofti* rural types found in Papua, East Nusa Tenggara, while *W. bancrofti*urban type commonly found in urban cities such as Bekasi, Tangerang, Pekalongan and Lebak. *B. malayi* found in Sumatra, Kalimantan, Sulawesi, and some islands in the Maluku. *B. timori* found in the islands of Flores, Alor, Rote Timor and Sumba [8].

Vector control such as environmental manipulation to eradicate marsh plants and sewer drain smoothly is one of the environmental management measures that can be done to empower the community. It can support government programs in order to achieve the target of elimination of filariasis by 2020.Prevention of filariasis can be done in three ways, namely the reduction of infectious reservoir, control vector (mosquito), and reduction of vector and human contact [9].

The Government has been implementing filariasis elimination programs since 2002 and Indonesia in 2020 is planned free filariasis. Efforts have been made to reduce the prevalence of filariasis are as follows: (a). eradication of mosquitoes; (b). efforts for the mass treatment of endemic areas with a number greater than 1 %, the transmission can be prevented by prevention programs filariasis mass drug administration (POMP filariasis); (c). environmental management by providing environmental engineering are expected to reduce the prevalence of filariasis in Indonesia through community empowerment [9].

DISCUSSION

Filariasis vector

In Indonesia filariasis is caused by three species of filarial worms, including *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, while the vector is mosquitoes. There are 23 known species of mosquitoes of the genus Mansonia, Anopheles, Culex, Aedes,and Armigeres that can serve as potential vectors of filariasis. Ten species of Anopheles mosquitoes have been identified as vectors of *W. bancrofti*rural type, while the vector of urban type is *Culex quinquefasciatus*. As for *B. malayi*, there are 6 (six) and Mansonia species to eastern Indonesia also *Anopheles barbirostris* [10].

Incidence of filariasis was found both in rural and urban areas, based on this in epidemiology filarial worms were divided into 6 types as follow [11]:

1. *Wuchereria bancrofti* urban type, which found in urban areas such as Bekasi, Tangerang, Pekalongan. The type has nocturnal periodicity, and transmitted by *Cx. quinquefasciatus* that breeds in household waste water.
2. *Wuchereria bancrofti* rural type, which found in rural areas outside Java, especially in Papua and East Nusa Tenggara. The type has nocturnal periodicity and transmitted by various species of the Anopheles and Culex.
3. *Brugia malayi* nocturnal type. Microfilariae are found in the peripheral blood at night. The type is transmitted by *Anopheles barbirostis* in rice fields
4. *Brugia malayi* subperiodic nocturnal type. Microfilariae are found in the peripheral blood on day and night, but are more common at night. The vector is *Mansonia spp*, which found in swamp areas.
5. *Brugia malayi* non-periodic type. Microfilariae are found in the peripheral blood both at night and during the day. *Mansonia bonneae* and *Mansonia uniformis* are the vector, which found in the jungle.
6. *Brugia timori* periodic nocturnal type. Microfilariae are found in the peripheral bloodat night. The vector, *An. barbirostris*, is found in the rice fields of East Nusa Tenggara, Maluku Tenggara.

In general, the life cycle of the worm species do not differ. Life cycle of the parasite occurs in the human body and the body of the mosquito. Adult worms (called makrofilaria) live in channels and lymph nodes, while his son (called microfilaria) is in the circulatory system.

Environmental factors

Environmental conditions greatly affect the distribution of filariasis cases and the chain of transmission [11]. In general, endemic area of *B. malayi* is areas with swamp forests, along rivers or other water bodies are covered with water plants. Endemic areas of *Wuchereria bancrofti* urban type are urban slums, densely populated and many puddles of dirty water as the habitat of the mosquito vector *Cx. quinquefasciatus*. Whereas endemic areas of *Wuchereria bancrofti* rural type is generally equal to the endemic area *B. malayi* [12].

Environment can become breeding places of mosquitoes. In general, the environment can be divided into the physical environment, biological environment and social environment, economic and cultural [12].

a. Physical Environment

Physical environment includes, among others, the climate, the geography, geological structure, temperature, humidity and so on. They are closely related to the life of a vector, and therefore contributes to the emergence of the sources of transmission of filariasis. The physical environment can create breeding and resting places of mosquitoes. Environment with swamp water plants and the existence of reservoir host (apes, monkeys and cats) effect on the spread of B. malayi sub nokturna periodic and non- periodic.

1. Temperature

   Temperature affects growth, life span and the presence of mosquitoes. The optimum temperature for mosquito ranged 25-30^0 C [13].

2. Humidity

   Humidity affects growth, life span and the presence of mosquitoes. Low humidity will shorten the life of the mosquito. Humidity affects the speed of breeding,

biting habits, rest, and others from mosquitoes. 60 % humidity level is the lowest limit to allow her mosquito. At high humidity and mosquitoes become more active more often biting, thus increasing the transmission [11].

3. Wind speed at the time of sunrise and sunset which is when mosquitoes flight into or out of the house, is one of the factors that will determine the amount of contact between humans and mosquitoes. Mosquito flight range (flight range) can be shortened or extended depending on the wind direction. Anopheles mosquito flight range is usually limited to no more than 2-3 km from the place perindukannya. When there are strong winds Anopheles mosquitoes can carry up to 30 km.
4. Rain
   Rain associated with the development of mosquito larvae into the adult form. The size of the effect depends on the type of rain, heavy rain, number of rainy days and the type of vector breeding species (breeding place).
5. Sunlight
   Sunlight effect on different species of mosquitoes. For example Aconitus more like a place to breed in the water and the sun shade. Other species do not like water with enough sun but prefers a shady spot, Effect of sunlight on the growth of mosquito larvae vary. *An. sundaicus* prefer shade, while*An.hyrcanus spp.*and *An. punctulatus spp* prefer open places, and An. barbirostris can live well in the shade and the light [13].

b. Biological environment

Biological environment may be a factor supporting the transmission of filariasis. Examples of biological environment is the presence of aquatic plants, puddles, swamps and bushes as the growth of the mosquito *Mansonia spp*. Mangrove plants, moss, algae and other plant variety can affect larval life because he can block out sunlight or protect against other living beings

The existence of various types of larvae -eating fish such as fish head tin (*Panchax spp*), Gambusia, tilapia, tilapia and others will affect the population of mosquitoes in an area. Besides the large livestock such as cattle, buffalo and pigs can reduce the number of mosquito bites on humans, if animals are not caged away from home; this depends on a bite of mosquitoes [11].

Vector control management fiariasis

Vector management for prevention of filariasis can be done through the identification of the type of mosquito species by breeding. Further identifying the time and place of biting mosquitoes. This is a disease vector species Anopheles, Aedes, Culex and Mansonia. There are the different types of species based on environmental conditions such as in the tropics vector *Culex quiquefasciatus* and *C. pipiens* in temperate areas. Brugia and Wuchereria always indicate the nature nocturnal periodicity of microfilaria in the peripheral blood, the vector bite only at night, but there are some such as Mansonia and Aedes species have diurnal nature. Based on this difference in the vector control measures can be done by [13]:

a. If transmission occurs by mosquitoes that bite at night in the house of the preventive measures that can be done is by spraying, use of residual pesticides, put wire netting, sleeping by using mosquito nets (better already dyed with pyrethroid insecticides),

using anti liniment mosquitoes (repellents) and clean up mosquito breeding places such as an open latrine, old tire, coconut shells and kill larvae with larvicides.

b. If found Mansonia as vectors in an area, the action taken is to clean the pools of water plants are a source of oxygen for the larvae.

Vector control

Vector control is the most important effort. In areas with a high endemicity level, it is important to know exactly bionomic of mosquito vectors, the prevalence and incidence of disease, and environmental factors that play a role in supporting the transmission in each region.

There are 3 types of vector control [14]:

a. Maintaining biological control with natural enemies
b. Chemically control using insecticides (however the cause of environmental pollution should be considered)
c. Control engineering aimed at reducing insect nests (breeding places) to perform environmental management, manipulation and modification of the environment Manipulation is a form of activity to produce states of the environment that are not favorable for vector multiply in breeding place. If the mudflats to do are clean the moss or water plants as vector breeding places.
   Modification is to permanently improve the quality of the environment, such as drying, stockpiling puddles, drainage maintenance, repair temporary landfills or end.
   Management is permanent (long-term).

Environmental management

Environmental management in an effort to break the life cycle of the vector can be done by neighborhood vector lives. Mansonia mosquitoes require special water plants as the egg attaches to the lower surface of leaves of host plants in the form of groups of 10-16 grains. The egg-shaped oval with one end tapered. Then, larvae and pupanya attached to the roots or stems of aquatic plants by using a hook. The hook tool, when the larvae are at the end of the siphon, while the pupa found on trumpet. So that, by means of the hook, either siphon or trumpet can deal directly with air (oxygen) in the air network aquatic plants [14]. Environmental management methods that can be done are eliminating the host - plants from the breeding grounds [15].

CONCLUSION

1. Environmental management as an effort to reduce the prevalence of filariasis can be focused to perform engineering environments such as manipulation and modification of the environment.
2. Life cycle of worms Brugia sp with sp vector can be decided by transmission chain environmental management is to eliminate breeding places of water plants.

REFERENCES

1. Depkes RI, *Epidemiologi Penyakit Kaki Gajah(Filariasis) di Indonesia*, Buku 2, Ditjen PPM danPLP, Jakarta, 2002
2. Depkes RI, Mengenal Filariasis, Direktorat Pengendalian Penyakit Bersumber Binatang, 2009

3. Kemenkes RI, 2009, Laporan Kasus Klinis Filariasis di Indonesia tahun 2000-2009, Subdit Filariasis, Kementrian Kesehatan (Kemenkes) RI, Jakarta,2009
4. Kemenkes RI, 2010, Rencana Nasional Program Akselerasi Eliminasi Filariasis di Indonesia, Subdit Filariasis &Scistosomiasis Direktorat P2B2, Ditjen PP &PL Kemenkes RI, 2010
5. Purwantyastuti, Pemberian Obat Massal Pencegahan (POMP) Filariasis, Filariasis di Indonesia, Buletin Jendela Epidemiologi Vol 1 Juli 2010, Pusat Data Surveilens EpidemiologiKemenkes RI, 2010
6. Kemenkes RI, Rencana Nasional Program Akselerasi Eliminasi Filariasis Di Indonesia, Subdit Filariasis & Shistosomiasis, Direktorat P2B2, Ditjen PP & PL, Jakarta 2010
7. Kemenkes RI, Rencana Nasional Program Akselerasi Eliminasi Filariasis Di Indonesia, Subdit Filariasis & Shistosomiasis, Direktorat P2B2, Ditjen PP & PL, Jakarta 2010
8. Susanna Dewi, Entomologi Kesehatan Buku 1, UI Press, Jakarta 2011
9. Mulyono, R.A. *Risk Factor Environment andBehaviour Influence The Occurance of Filariasis (Case Study in Area Pekalongan)*. Jurnal Bina Sanitasi Volume 1, No 1 Desember 2008 : ISSN 2085-0190.
10. Depkes RI, Mengenal Filariasis , Direktorat Pengendalian Penyakit Bersumber Binatang, 2009
11. WHO, Hand Book For Integreted Vector Management, 2012
12. Depkes RI, P*edoman Program Eliminasi Penyakit Kaki Gajah (Filariasis) di Indonesia*, Buku 1, Ditjen PPM dan PLP, Jakarta, 2002
13. Depkes RI, *Ekologi Dan Aspek Perilaku Vektor*, Dit.Jen. PP & PL. Depkes RI, 2007
14. Depkes RI, *Desaku Bebas Penyakit Kaki Gajah(filariasis)*,Buku 7; Ditjen PPM dan PLP, Jakarta, 2002
15. Yudhastuti R , Pengendalian Vektor dan Rodent, Pustaka Melati, Surabaya, 2011

RESISTANCE STATUS OF *Aedes aegypti* L. AGAINST ORGANOPHOSPHATE LARVACIDE (TEMEPHOS), ORGANOPHOSPHATE (MALATHION) AND PYRETHROID (SIPERMETRIN) INSECTICIDE IN THE GEDONGKIWO VILLAGE, MANTRIJERON SUB DISTRICT, YOGYAKARTA

Tri Wahyuni Sukesi
Public Health Faculty University of Ahmad Dahlan, Yogyakarta
Correspondence: yons_by@yahoo.com

ABSTRACT

Background: Gedongkiwo Village had highest number of dengue patients in the year 2010. Health Center control the mosquito vector *Aedes aegypti* L. by provision of temephos, malathion, and sipermetrin. However, the results remain not as expected. The purpose of this study was to determine the resistance status of *Aedes aegypti* L. to temephos, malathion, and Sipermetrin.
Methods: This was a descriptive study through laboratory examination. The object of this research is mosquito larvae *Aedes aegypti* L. instar III-IV from the Gedongkiwo Village, taken by simple random sampling from RW 2, 6 and 9. Variable in this study was the resistance of *Aedes aegypti* L. larvae instars III and IV. Qualitative analysis was done by comparing the color intensity of the sample homogenate with color intensity of positive and negative controls homogenate. Quantitative results of the analysis carried out by reading the absorbance value (AV) using ELISA reader at $\lambda = 450$ nm with the estimation cut off point. Mosquito mortality was observed and recorded daily during the study.
Results : This study shows that the mosquito *Aedes aegypti* L. derived from the Village Gedongkiwo Mantrijeron Yogyakarta District has a high resistance state by 84, 53% against temephos, the status of 100% high resistance to malathion, and resistance status was a mortality rate of 84% against sipermetrin.
Conclusion: Mosquito *Aedes aegypti*L. in the Village Gedongkiwo, Mantrijeron, Yogyakarta has encountered high resistance to temephos and malathion, but still middle resistance tosipermetrin.
Keywords: *Aedes aegypti*L., insecticides, larvacide, resistance status

BACKGROUND

Dengue Hemorrhagic Fever (DHF) is a contagious disease that is still a public health problem in Indonesia, often appears as an extraordinary events and cause panic in the community because it spreads rapidly and can cause death [1]. Clinical symptoms of DHF that goes on continuously for 2-7 days and bleeding manifestations are usually preceded by sightings of the typical signs such as red spots (petechia) on the body of the patient. In severe cases,the patient may go into shock and die [2].

DHF is caused by dengue virus and is transmitted through the bite of Aedes aegypti L. which is the main vector of DHF. Dengue virus belongs to a group B Arthropod Borne Virus (Arboviruses) are now known as the genus Flavivirus, family Flaviviridae, and have 4 types of serotype, the DEN-1, DEN-2, DEN-3, and DEN-4.

Serotype DEN-3 was the predominant serotype and assumed many showed severe clinical manifestations [3].

DHF and Dengue Fever is endemic in tropical countries [4]. The attack of dengue in Indonesia was first reported in 1968 although at times it has not been proven in practice. Then, in 1970 there was an attack of dengue in Jakarta. Attacks occurred in almost all provinces in Indonesia reached 19,000 people over the 1.8% mortality rate, or about 342 people [5].

According to data obtained from the hospital information system in Indonesia in 2009, especially in the province of Yogyakarta reported there were 2013 cases of dengue fever in the hospital inpatient, 25 were killed and as many as 60 cases by the number of outpatient visits of 110. Among the five districts located in the province of Yogyakarta, Yogyakarta city has the highest number of dengue cases in the year 2009 as many as 1178 new cases and 9 of them died [6].

There are 5 ways to control vector, ie by chemical, genetic, biological, radiation, and mechanical/environmental management. Dengue vector control has been widely applied, as it is the most effective way to help break the chain of transmission of Dengue [7]. In Indonesia and many other developed countries, efforts to control *Aedes aegypti* L. highly dependent on the chemical control by using insecticides. This can be seen in the use of class organophosphates (temephos and malathion) has been in use since the 1970s [8].

Theoretically, the use of larvacide in a long time will cause resistance. The emergence of resistant strains of insects is triggered by prolonged exposure. This happens because the mosquito *Aedes aegypti* L. and the others of dengue vector are able to develop immunity to the larvacide systems are often used. In many parts of the world has been widely reported the resistance of larvae of *Aedes aegypti* L. against larvacide and insecticides, among which are occurred in Brazil, Bolivia, Argentina, Venezuela, Cuba, French Polynesia, the Caribbean and Thailand [9]. In a previous study conducted by Sukesi [10], biokemis obtained test results show that the larvae of *Aedes aegypti* L. in the Gedongkiwo Village overall showed a decrease in the status of resistance to organophosphate compounds (temephos and malathion) from susceptible or resistant (SS) 33% into susceptible or resistant to moderate (RS) by 44%.

Besides the use of organophosphate group, one of the efforts undertaken to prevent dengue is to fogging or fumigation with insecticide made active use Sipermetrin and malathion to kill mosquitoes *Aedes aegypti* L. adulthood. Evaluation of control efforts that have been done so far is effective at killing and reduce the population of Aedes aegypti L as vectors or dengue mosquito transmission.

Gedongkiwo Village is one of the villages which tops the list for the highest number of dengue patients in the year 2010. Based on the data obtained from the Department of Health Yogyakarta, the year 2010 recorded the number of dengue cases that occurred in the Gedongkiwo Village as many as 76. Gedongkiwo Village is located in the district of Yogyakarta Mantrijeron and are in the middle of the city of Yogyakarta who have conditions that are densely populated, less orderly and slums. The number of water reservoirs in every home and a location close to the bustling transport flow can increase the chances of the development of *Aedes aegypti* L. thus increasing the number of dengue cases in the community. Until recently, dengue prevention is still resting on the success of vector control. Many years of health centers in the Gedongkiwo Village backed government has attempted to prevent and eradicate dengue by controlling the

mosquito vector *Aedes aegypti* L. However, the results are still not as expected. The results can be seen from the number of dengue cases in the Village Gedongkiwo still remain high. Use larvacide and insecticide in a long time is possible has led to reduced susceptibility to mosquito *Aedes aegypti* L. that can lead to resistance.

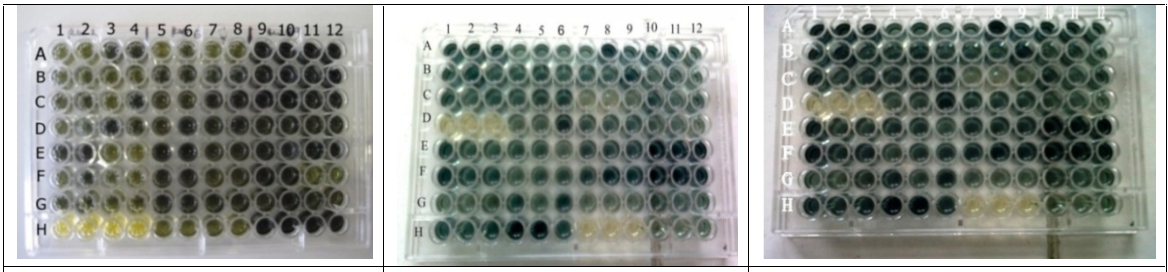
METHODS

The method is descriptive research with laboratory testing. The object of this research is mosquito larvae *Aedes.aegypti* L. instar III-IV from the Gedongkiwo Village Mantrijeron Sub District Yogyakarta City taken by simple random sampling that at RW 2, RW RW 6 and 9.

Variable in this study is a single variable, that is the resistance of larvae of *Aedes aegypti* L. instars III and IV. Analysis of survey data conducted qualitative and quantitative, as well as by looking at the mosquito mortality. Qualitative analysis of the results is done by comparing the color intensity of the sample homogenate with color intensity of positive and negative controls homogenate, while the quantitative results of the analysis carried out by reading the absorbance value (AV) using ELISA reader at λ = 450 nm with the estimation of the cut off point.

RESULTS

Qualitatice biochemical test



(a)	(b)	(c)

Picture 1. The results of biokemis test increased Esterase Nonspesific Enzym (a dan b) and Mono Oxigenase Nonspesific Enzym (c) of *Aedes aegypti* L larvae against temephos (a), malathion (b), sipermetrin (c) in the Gedongkiwo Village, Mantrijeron Sub District, Yogyakarta City.

Description:

1. Rows A-H containing larvae of Aedes aegypti L. homogenates from District Gedongkiwo Mantrijeron District of Yogyakarta (except Column D rows 1-3, columns 7-9 rows H and H lines 10-12).
2. Columns 1-3 line D contains larvae of Aedes aegypti L. homogenates are still susceptible (negative control)
3. Columns 7-9 lines of H contains a reagent
4. Columns 10-12 H lines contain larvae of Aedes aegypti L. homogenates Laboratory of Parasitology Medical Faculty Gadjah Mada University which is resistant (positive control)

Quantitative biochemical test

Table 1. Determination of the status of resistance in each replicate *Aedes aegypti* L. mosquito againts larvacide organophosphates (temephos) from Gedongkiwo Village, Mantrijeron Sub District, Yogyakarta

	1	2	3	4	5	6	7	8	9	10	11	12
A	√	X	√	√	X	√	X	#	√	√	√	√
B	√	√	√	√	√	√	#	X	√	√	√	√
C	√	√	√	√	√	√	√	√	√	√	√	√
D	√	√	√	√	√	√	√	√	√	√	√	√
E	√	√	X	√	√	√	√	√	√	#	√	√
F	#	√	X	#	√	√	√	√	√	√	X	X
G	√	√	√	√	√	√	√	√	√	√	√	√
H	X	X	X	X	X	X	X	X	√	√	√	√

Vulnerable (SS)= X; Medium (RS) = #; Resistant (RR) = √

Table 2. Determination of the status of resistance in each replicate mosquito *Aedes aegypti* L. againts organophosphate insecticides (malathion) from Gedongkiwo Village, Mantrijeron Sub District, Yogyakarta

	1	2	3	4	5	6	7	8	9	10	11	12
A	√	√	√	√	√	√	√	√	√	√	√	√
B	√	√	√	√	√	√	√	√	√	√	√	√
C	√	√	√	√	√	√	√	√	√	√	√	√
D	X	X	X	√	√	√	√	√	√	√	√	√
E	√	√	√	√	√	√	√	√	√	√	√	√
F	√	√	√	√	√	√	√	√	√	√	√	√
G	√	√	√	√	√	√	√	√	√	√	√	√
H	√	√	√	√	√	√	X	X	X	√	√	√

Vulnerable (SS) = X; Medium (RS) = #; Resistant (RR) = √

Table 3. Determination of the status of resistance in each replicate mosquito *Aedes aegypti* L. pyrethroid insecticides (sipermethrin) of Gedongkiwo Village, Mantrijeron Sub District, Yogyakarta

	1	2	3	4	5	6	7	8	9	10	11
A	X	X	X	X	X	X	X	X	X	X	X
B	X	X	X	X	X	X	X	X	X	X	X
C	X	X	X	X	X	X	X	X	X	X	X
D	√	√	√	X	X	X	X	X	X	X	X
E	X	X	X	X	X	X	X	X	X	X	X
F	X	X	X	X	X	X	X	X	X	X	X
G	√	X	√	X	X	X	X	X	X	X	X
H	X	X	X	X	X	√	√	√	X	X	X

Susceptibility test

Table 4. Distribution and Frequency of Resistance Test Results In Susceptibilities *Aedes aegypti* L. Mosquito Against Organophosphate Insecticides (Malathion) in the Village District Gedongkiwo Mantrijeron Yogyakarta in 2012

Perlakuan								
	Malathion 0.8%				Kontrol			
	Hidup	%	Mati	%	Hidup	%	Mati	%
	18	72	7	28	20	80	5	20

Based on susceptibility test results above can be seen the resistance status of *Aedes aegypti* L. mosquito of Gedongkiwo Village. Mosquito mortality rate 25% lower than 80%, then the mosquito Aedes aegypti L. derived from the Gedongkiwo village, Mantrijeron Sub District, Yogyakarta was resistant to malathion.

Table 5. Distribution and Frequency of Resistance Test Results In Susceptibilities *Aedes aegypti* L. Mosquito Against Pyrethroid Insecticides (Sipermetrin) in the Village District Gedongkiwo Mantrijeron Yogyakarta in 2012

Treatment							
Sipermetrin 0,05%				Kontrol			
Living	%	Dead	%	Living	%	Mati	%
4	16%	21	84%	22	88%	3	12%

Based on susceptibility test results above can be seen the resistance status of *Aedes aegypti* L. mosquito of Gedongkiwo Village. 21 mosquito mortality rate 84% are within the 80-98% in the medium to insecticides Sipermetrin said.

DISCUSSION

Biochemical Test

Biochemical Testing performed to determine the range and distribution of values Absorbance Value (AV) and the color changes that occur during the biochemis process

to increased Non-Specific Esterase Enzyme activity and Mono Oxigenase Enzyme larvae of *Aedes aegypti* L. samples Gedongkiwo Village against organophosphates larvacides (temephos), organophosphate insecticides (malathion), and pyrethroid insecticides (sipermetrin).

Temephos

The test results showed that the change of color in all test samples homogenates of larvae of *Aedes aegypti* L. from Gedongkiwo Village is colorless (clear) to dark blue and some samples have changed color to a light blue-green. Most of the test larvae homogenates, namely *Aedes aegypti* L. from Gedongkiwo Village produce dark navy color approaching homogenates positive control and only a small proportion of larvae homogenates test that produces pale blue-green color approaching negative control homogenates.

The color change and the high value of AV in the test sample showed most *Aedes aegypti* L. from Gedongkiwo Village has increased *non-specific esterase enzyme*. Based on predetermined criteria results obtained 9.52% *Aedes aegypti* L. status remains fragile, 5.95% *Aedes aegypti* L. status 5.95% *Aedes aegypti* L. status are less resistance and 84.53% *Aedes aegypti* L. resistant status. So it can be said that the mosquito *Aedes aegypti* L. in the Gedongkiwo Village, Mantrijeron Sub District, Yogyakarta has encountered resistance to temephos.

Malathion

The test results showed a color change in all test samples homogenates larvae of *Aedes aegypti* L. derived from the Gedongkiwo Village. Whole homogenates of larvae of *Aedes aegypti* L. from Gedongkiwo Village produce blue color the same color as the positive control larvae homogenates, in fact some homogenates were darker than the positive control homogenates.

The results were measured using Elisa reader with a wavelength of 450 nm. The measurement results obtained turned out to produce a high grade AV and 100% test larvae homogenates, namely *Aedes aegypti* L. from Gedongkiwo Village Mantrijeron Sub District Yogyakarta resistant to malathion.

Sipermetrin

It is known from the test results showed that the change of color in all homogenates test samples of larvae of *Aedes aegypti* L Gedongkiwo Vilage, which is of no color (clear) to light blue-green and a few sample turns blue dark blue. Most of larvae test homogenates from Gedongkiwo Village *Aedes aegyti* L produces a blue color close to the color blue.

The results of this study Gedongkiwo Village, mostly showing a light blue color as measured using the ELISA reader at a wavelength of 655 nm turned out to produce value Absorbance Value (AV) is low. The percentage of the overall status of Vulnerable as much as 100%.

The color changes that the low value of AV in the test samples showed mostly *Aedes aegypti* L in the Gedongkiwo Village has experienced an increase in *mono oxigenase enzyme*. In this study sample was taken at random, so that can know the status of the Gedongkiwo Village resistance based on the criteria that have been set

obtained results 100% *Aedes aegypti* L mosquito status is vulnerable, so it can be said that the Aedes aegypti L mosquito in Gedongkiwo Village is still in circumstances susceptible to insecticides (Sipermetrin). At the time of the mechanisms of resistance in the mosquito Aedes aegypti L with the results due to the use of insecticide susceptible (Sipermetrin) recently conducted its use in the Gedongkiwo Village.

The occurrence of resistance to the mosquito *Aedes aegypti* L. the larvacide organophosphates (temephos) and organophosphate insecticides (malathion) most likely a result of long-term use and are not regular and larvacide and insecticide doses that are not fixed. Ongoing mechanism of resistance in the mosquito *Aedes aegypti* L. most likely caused by the length of use larvacide organophosphates (temephos) and organophosphate insecticides (malathion) and mosquitoes also frequent contact with these insecticides.

Based on interviews with environmental health officers in each region and information from Mantrijeron Health Center, in the Gedongkiwo Village is indeed more common in dengue cases compared to other villages, so abatisasi more often implemented. Abatisasi higher frequencies can be more encouraging resistance in populations of *Aedes aegypti* L. in the Gedongkiwo Village. In addition, the use of temephos and malathion over the years and Sipermetrin recently used can also trigger the development of resistance.

An increase in *nonspecific esterase enzyme* activity and *mono oxigenase enzyme* on some members of the population tested could be triggered by three groups of insecticides, the organophosphates, carbamates and pyrethroids. The test results with the substrate α-naphthyl acetate showed only one group that triggered the emergence of insecticide resistance genes in populations observed. The results indicate the emergence of resistance genes occurs in the substrate α-naphthyl acetate. These results indicate the incidence of resistance because esterase enzyme activity only happened because one group of organophosphate insecticides, carbamate or pyrethroid saja [11].

The main factors affecting the nature of resistance include genetic, biological, and operational. Genetic factors include the amount of a specific gene as a control resistant (R-genes) either dominant or recessive, including esterase II and III genes regulating enzymes work to neutralize organophosphate esterase. The existence of genes controlling this will affect the vulnerability status of *Aedes aegypti* L. against organophosphate.

Biological factors here more emphasis on the properties owned by the mosquitoes which includes regeneration, marriage patterns and the expiration of the development of each generation and the behavior of the mosquito itself. While operational factors that affect the resistance mechanisms include the chemicals used and their application in the field (the way the application, frequency, proper dosage and duration of use) [10].

Use larvacide repeatedly for a long time and not regularly can act as agents of natural selection population that will make the mosquitoes that have resistance genes to survive and be passed on to the next generation. As a result, the percentage of resistant mosquitoes will continue to grow, while the susceptible mosquitoes will be eliminated because of larvacide. In the end, the use of larvacide become ineffective as the number of resistant mosquitoes far more than the mosquitoes rentan [8].

Susceptibility Test

Susceptibility testing performed to determine the resistance status of *Aedes aegypti* L. derived from the Gedongkiwo Village to organophosphate insecticides (malathion) and pyrethroids insecticides (Sipermetrin), by looking at the death rate of mosquitoes exposed to malathion and Sipermetrin. The mosquito *Aedes aegypti* L. the test sample is the mosquito *Aedes aegypti* L. were full of blood, so that in this test mosquitoes that die not because of hunger but really because it is caused by exposure to malathion and Sipermetrin.

In susceptibility testing organophosphate insecticides (malathion) occurred two treatment replications. The first case of death Deuteronomy 100%, well exposed to malathion and the control. This happens because at the time of the study room humidity is not considered, thus resulting in the death of the mosquito. For the second replay manunjukkan different results, the mosquito *Aedes aegypti* L. exposed to malathion 0.8% of the living as much as 18 mosquitoes (72%) and dead mosquitoes were 7 (28%). As for the mosquito *Aedes aegypti* L. the control of the living as much as 20 mosquitoes (80%) and were dead by 5 mosquitoes (20%).

Testing of susceptibility to pyrethroid insecticides (Sipermetrin) in the second replay shows the results of the mosquito *Aedes aegypti* L Sipermetrin exposed to 0.05% of the living as much as 4 mosquitoes (16%) and were dead by 21 mosquitoes (84%), while for the mosquito Aedes aegypti L the control of mosquitoes that live by 22 (88%) and as many as 3 dead mosquitoes (12%).The results of susceptibility testing showed that the mosquito *Aedes aegypti* L. the beraasal from Gedongkiwo Village was resistant to malathion, and Sipermetrin. Susceptibility testing is further strengthens the previous test results also showed biokemis same results.

The use of insecticides, can act as agents of natural selection population that will make the insect resistant gene has remained alive and will be handed down to the next generation. As a result, the percentage of resistant insects will continue to grow, while the susceptible insects will be eliminated by insecticides. In the end, the use of insecticides to be ineffective because the number of insects far more resistant than susceptible insects [8].

CONCLUSION

Aedes aegypti L. mosquito derived from Gedongkiwo Village as a whole has experienced resistance against organophosphates larvacides (temephos) 84.53% with high resistance and 5.95% were resistant status.*Aedes aegypti* L. mosquito derived from Gedongkiwo Village as a whole has experienced resistance against organophosphates insecticides (malathion) 100% with high resistance to test biokemis and mortality of 28% which indicates resistance to susceptibility testing. *Aedes aegypti* L. mosquito derived from Gedongkiwo Village as a whole has experienced resistance against pyrethroid unsecticides (sipermetrin) with middle resistance status with a mortality rate 84% for susceptibility testing and 100% susceptible to biokemis test.

REFERENCES

1. Depkes RI, 2008, Profil Kesehatan Indonesia Tahun 2007, Departemen Kesehatan RI, Jakarta, Hal. 85.

2. Sutanto I, Ismid IS, Sjarifuddin PK, Sungkar S. 2008, Parasitologi Kedokteran, Fakultas Kedokteran UI, Jakarta, Hal. 256-266, 276, 280, 282, 286.
3. Depkes RI, 2001, Tata Laksana Demam Berdarah Dengue di Indonesia, Direktorat Jenderal Pemberantasan Penyakit Menular dan Penyehatan Lingkungan Departemen Kesehatan RI, Jakarta, Hal.1, 13.
4. Sitorus H. 2008, Deteksi Resistensi Larva Aedes aegypti (LINN.) dan Aedes albopictus (SKUSE) Terhadap Malathion dan Temephos serta Pemetaan Kerawanan Infeksi Demam Berdarah Dengue di Kecamatan Sukarame Kota Palembang Sumatera Selatan", Tesis, Pascasarjana Fakultas Kedokteran UGM, Yogyakarta.
5. Sembel, D. T., 2009, EntomologiKedokteran, Andi, Yogyakarta, Hal 53, 61, dan 63.
6. Depkes RI, 2010, Sistem Informasi Rumah Sakit di Indonesia, Direktorat Jenderal Pelayanan Medik, Jakarta
7. Soegijanto, S., 2006, Demam Berdarah Dengue, Airlangga University, Surabaya, Hal. 2, 254, 247-251, 254-256.
8. Ahmad I, Astari S, Rahayu R, Hariani N. 2009. Status Kerentanan Aedes aegypti (Diptera: Culicidae) Pada Tahun 2006-2007 Terhadap Malathion di Bandung, Jakarta, Surabaya, Palembang dan Palu. Biosfera2009;26(2):85-86.
9. Gafur, A., Mahrina, Hardiansyah, 2006, "Kerentanan Larva Aedesaegyptidari Banjarmasin Utara TerhadapTemefos", BIOSCIENTIAE, Vol 3 No 2 Juli 2006, Program StudiBiologi FMIPA ULM, Kalimantan Selatan, Hal.74.
10. Sukesi, T. W., 2007, "Kerentanan Nyamuk Aedes aegypti L. Terhadap Senyawa Organofosfat Temephos dan Malathion di Kelurahan Wirobrajan Kecamatan Wirobrajan Yogyakarta", Kes Mas2007;1(1): 24-25.
11. Nusa, R., Ipa, M., Delia, T., Santi, M., 2008, "Penentuan Status Resistensi Aedes aegypti dari Daerah Endemis DBD di Kota Depok Terhadap Malation" Buletin Penelitian Kesehatan2008;36(1):20-24.

COMPARATIVE EFFECTIVENESS OF PYRIPROXYFEN AND METHOPRENE TO *Aedes aegypti* LARVAE FROM WEST BANJARMASIN

Istiana
Departement of Parasitology, Medical Faculty of Lambung Mangkurat University
Correspondence: istiana_aribudi@yahoo.com

ABSTRACT

Background: Current larvicide used to control *Aedes aegypti* is temephos, although some studies have reported the presence of resistance. WHO recommended new larvicides contain active pyriproxyfen and methoprene from Insect growth Regulator (IGR) group as a substitute of temephos. This study aimed to determine the effectiveness pyriproxyfen and methoprene as larvicides and the time required in killig 50% larvae of *Aedes aegypti* from West Banjarmasin.
Method: This was an experimental study with post test only with control group design. Doses of pyriproxyfen were 0.5, 0.1, 0.05, 0.01, 0.008, and 0.005 ppm. Doses of methoprene were 0.01, 0.05, 0.1, 0.5, 1, and 1.5 ppm. Each treatment group was exposed to *Aedes aegypti* larvae. Larval metamorphoses and mortality were followed for 15 days. Analysis data used independent t test and probit.
Results: The result showed that pyriproxyfen effectively killed the larvae by 70% after seven days of exposure, while methoprene kills 50% in eight days. The optimum concentration to kill 50% of larvae (LC_{50}) for pyriproxyfen was 1.009ppm, while methoprene was 1.34 ppm. Test results of statistical analysis with Independent T test showed a significance different in killing larvae between pyriproxyfen and mehoprene ($p=0.039$).
Conclusion: Pyriproxyfen is more effective than Methoprene in killing larvae of *Aedes aegypti* from West Banjarmasin.
Keywords: effectiveness, pyriproxyfen, methoprene, *Aedes aegypti*

INTRODUCTION

Dengue Hemorrhagic fever (DHF) is an infectious disease that is still a public health problem in Indonesia, even likely to become endemic [1]. The case of DHF in 2012 is 90.245 cases recorded with the deaths of as many as 900 people. In South Kalimantan, the case of DHF is 1216 cases with the number of patients who died reached 19 cases (case fatality rate/CFR 1.5%). This number increased 3 fold compared to the year 2011 which is just 400 cases with the number of deaths are 7 people. Banjarmasin is the third most case with 56 cases. However, the cases who died reached 3 persons (CFR 5.3%) [2].

Dengue Haemorrhagic fever can only be controlled with vector eradication because the drug and vaccine for it has not found [1]. Control of the DHF can be done by eradicating adult and larvae of *Aedes aegypti* [3,4]. The adult mosquito eradication can be done by fogging and eradication of larvae can be done with *GERAKAN 3M* plus and using abate powder. However, long-term use of temephos has lower sensitivity against larvae [5-7]. The research of Gafur *et al* in North Banjarmasin showed a

decrease susceptibility of larvae to temefos [8]. Istiana *et al* also mentions that in West Banjarmasin showed a decrease in the susceptibility of larvae of *Aedes aegypti* to abate powder [9].

World Health Organization (WHO) has recommended the use of other insecticides as an alternative in dengue endemic areas that have resistance for temephos or have used temephos in the long time. One of the WHO recommendations is the use of Insect Growth Regulator (IGR) such as methoprene and pyriproxyfen [10].

The purpose of this study was to determine the effectiveness of larvicides pyriproxyfen and methoprene against *Aedes aegypti* larvae in the district of West Banjarmasin, South Kalimantan. This study is expected to provide scientific information as well as the basic for policy implementation on mosquito control program in Banjarmasin.

METHOD

This was an experimental study with Posttest-Only with Control Group Design. Subjects were larvae of *Aedes aegypti* Instar III-IV in second generation of colonization in Parasitology Laboratory, Medical Faculty of Unlam Banjarmasin. Independent variable wass Pyriproxyfen dose of 0.5 ppm, 0.1 ppm, 0.05 ppm, 0.01 ppm, 0.008 ppm, 0.005 ppm. While Methoprene dose was 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 1 ppm, and 1.5 ppm. Dependent variable was the *Aedes aegypti* larval mortality after administration of larvicides was followed for 15 days. Procedures used in this study was in accordance with the method set by WHO for experiments in the laboratory and the data obtained were analyzed using Probit Analyze with SPSS.

RESULTS

Results of research to determine effectiveness of IGR (pyriproxyfen and methoprene) can be seen in the following figure. Observation of larvae mortality and morphogenesis performed for fifteen days to determine the time to reach 70% or more larval mortality.

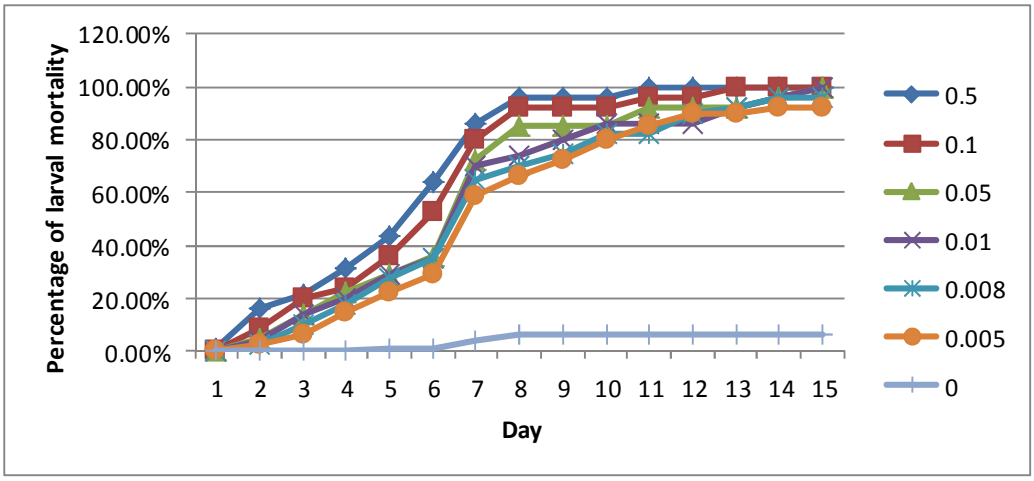


Figure 1. Percentage of Larvae of *Aedes aegypti*Mortalityfrom West Banjarmasinafter ExposuretoPyriproxyfen.

Figure 1 showed that the average percentage of dead larvae in the control group at 0% until day 4, and only 6.25% until day 15. This means development of mosquitoes in control group was well enough that larvae can develop into pupae and adult mosquitoes. The percentage of deaths in control group showed that deaths in other groups was purely caused by given larvicides. Because in this study the control larvae showed the average percentage mortality after 24 hours of exposure is 0% or no more than 20%, then itd oes not need to be corrected with Abbot Formula [11].

Statistical analysis by Kruskal Wallis test showed that all groups of dosage were differences in larval mortality (p=0.019). This is according to tests performed by Miller et.al. who also showed significant difference of larval mortality in the dosage of groups (p=0.001) [10]. The result of Mann-Whitney test to determine dose groups had significant differences, indicated that all groups were different ($p<0.05$). This significant difference can be seen in the 0.5 ppm dose group with the control group (p=0.001), 0.1 ppm with control (p=0.005), 0.05 ppm with controls (p=0.007), 0.01 ppm with controls (p=0.007), 0.008 ppm with control (p=0.010), and 0.005 ppm with controls (p=0.010). Subsequent data analysis using probit analysis to determine the dose required to obtain 50 % mortality and 90 % of the test larvae as in Table 1.

Table 1. Probit analysis results for Dose Required to Kill 50 % (LC 50) and 90 % (LC 90) Larvae Test

Larval mortality (%)	Doses (ppm)
10	0,314
20	0,553
30	0,735
40	0,872
50	**1,009**
60	1,146
70	1,294
80	1,466
90	**1,704**
95	1,901
99	2,271

The results with various concentrations of the administration methoprene to *Aedes aegypti* larvae are shown in Figure 2. It shows that in the control group, the average percentage of larvae on the eighth day mortality of 6.25 % to 7.5 % on day 15, so it does not need to be corrected using the formula abbott. Abbot formula is used if the percentage of control group mortality of more than 20 % in 24 hours of observation.

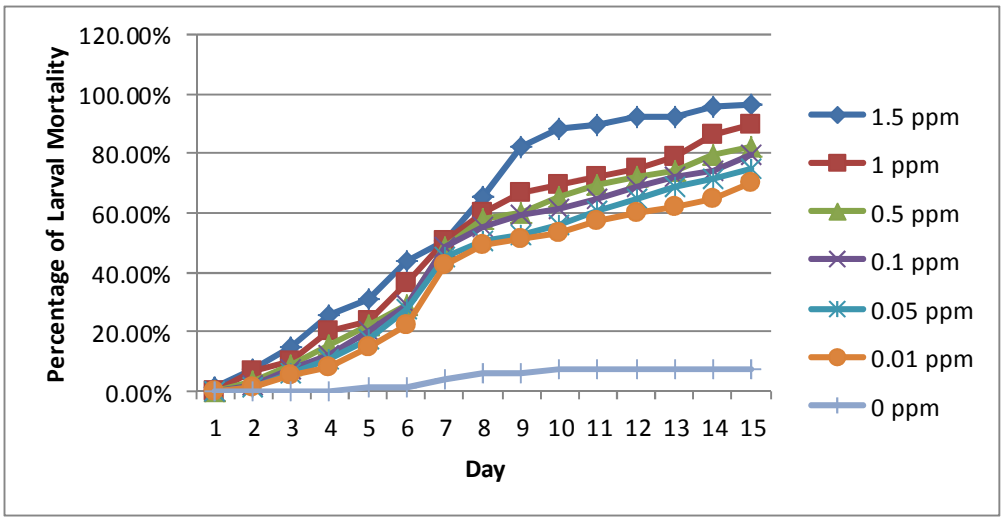


Figure 2. Percentage of Larvae of *Aedes aegypti*Mortalityfrom West Banjarmasin after Exposureto Methoprene.

Analyzed with Kruskal Wallis test showed the results of p=0.040, which means that there are significant differences between treatment groups. Further to determine significant differences between treatment groups, using the Mann-Whitney test. The results obtained indicate that there are several groups of p<0.05. This means that there are significant differences in the treatment groups between the control group with the group with a concentration of 0.05 ppm, 0.1 ppm, 0.5 ppm, 1 ppm and 1.5 ppm.

Probit analysis is then performed to determine the dose required to kill 50 % and 90 % larval test with results as shown in Table 2.

Table 2. Probit analysis results for Methophren Dose required Killing 50 % (LC 50) and 90 % (LC 90) Larvae of *Aedes aegypti*

Larval mortality (%)	Doses (ppm)
10	0,445
20	0,675
30	0,835
40	0,997
50	**1,342**
60	1,461
70	1,954
80	2,257
90	**2,704**
95	2,901
99	3,178

It shows that the LC50 for Methophren in this study was 1.342 ppm and the LC90 is 2.704 ppm. It means to kill larvae by 50 % and 90 % of the required dose.

DISCUSSION

Figure 1 shows that not all doses tested had an average percentage of larval mortality trials more than 70 % after 7 days, whereas at the highest dose of 0.5 ppm and 0.1 ppm indicate the average percentage of larval mortality trials reached more of 80 % and 86%. In the last days, all dose shows the average percentage of dead larvae reached more of 90% and for dose of 0.5 ppm and 0.1 ppm reached 100%. These results indicate that the percentage of larval mortality increased along with concentration or dose of larvicide and the length of time observation increased.

Based on the calculation criteria for larvicidal efficacy, as an active ingredient pyriproxyfen, after 24 hours of observation, it showed that all doses tested did not meet the criteria of efficacy due to larval mortality rate is below 70 % [12]. This is because the nature of active ingredient pyriproxyfen does not kill larvae directly, but inhibits development of larvae into pupae and adult mosquitoes. Pyriproxyfen that is in the water easily penetrates the skin mosquito larvae then enter into haemolymph. Pyriproxyfen in the haemolymph caused corpusallatum not produce juvenile hormone which caused the larvae do not develop into pupa or mosquitoes [13]. This was proven by observation until day 15, at all doses tested there was no change in the development of larvae into adult mosquitoes. This is very different from the control groups where the most of larvae change into adult mosquitoes.

Result of this study have similarities with research conducted by Munif, where the IGRA deal with active ingredient Pyriproxyfen, at dosage of 0.01 ppm has ability to inhibit growth of larva into mosquitoes reached 100 % after 1 week of exposure [14]. Although in this study dosage of 0.5 ppm inhibited 100 % of larvae growth on day 11, and at dosage 0.1 ppm on day 13.

Table 1 reveals that the dose required killing 50% of larvae of *Aedes aegypti* in this study was 1,009 ppm, while the dose required to kill 90% of test larvae was 1,704 ppm. Pyriproxyfen has a unique pattern of activity and influence on physiology, morphogenesis, reproduction, and especially insect embryogenesis metamorphosis that has properties such as *Aedes aegypti* mosquito is the primary vector of dengue fever [13]. Although larvicidal active ingredient pyriproxyfen takes time to kill mosquito larvae and pupae, pyriproxyfen has a long residual life according to tests performed by the Munif [14], at dosage 0.01 ppm pyriproxyfen can inhibit the growth of *Aedes aegypti* larvae into adult mosquitoes by 100% until fifth of week.

Percentage of larval mortality at various doses methoprene test increased with increasing concentrations of the test. This is showed in figure 2. Based on the results of the study, the average percentage rate larval mortality during the 24 hour at 0.41 % due to death on the first day it just occurred in the group treated with a dose of 1.5 ppm. On the eighth day of observation, the average percentage of larval mortality test all doses of more than 50 % except at doses of 0.01 ppm, and the highest concentrations of his death only 65 %. This percentage increased with the length of observation time, where at day 15, the average mortality in all treatment groups of more than 70 %, and reached 96.5 % at the 1.5 ppm dose group.

Increased mortality in line with the length of time due to the nature of the active ingredient methoprene kills larvae not directly, but inhibits the development and cause the death of the pupal stage and growth failure of adult mosquito's stage. This is according to research conducted Braga et al that the greatest number of percentage

mortality of larvae fed methoprene, found in the pupa stage, not on stage of larvae [15]. The same thing also expressed by Wu et al, that the increasing concentration of methoprene the percentage pupal mortality also increased [16].

However, because this is the nature of larvicides as a barrier to development, so that the higher concentration is given, then the percentage of deaths would be even greater. This is supported by Braga et.al. study [17], Wu et.al [16] and Pranoto [18] which shows percentage pupal mortality reached 100 % in the third week after treatment. It can support that methoprene inhibit larval development and kills pupa stage. Because on the fifth day, the control group there were a lot of mosquitoes mature and grow upon the eighth day. However, none of the treatment groups of larvae into adult mosquitoes up to eight days.

Methoprene is a larvicide that serve as artificial hormones which regulate growth-stage larvae (IGR-Insect Growth Regulators). Mechanism of action of this hormone does not kill larvae directly but inhibits the formation cytine during larval grow than disrupt the process of shape change into pupae and mosquitoes (inhibitor development), so it cannot grow into pupae of mosquitoes or mosquitoes can grow into, but not normal [19].

To compare the effectiveness of pyriproxyfen with methoprene performed by independen t Ttest analysis. The analysis showed p=0.039, which means that there is a significant difference between the effects larvicides produced by pyriproxyfen with methoprene. If seen from the LC50 and LC90 between pyriproxyfen with methoprene, it appears that pyriproxyfen better than methoprene.

In the life cycle of the mosquito phase of growth the reare egg, larva, pupa and adult. This growth phase requires growth regulator hormone called juvenile hormone, an enzyme that plays a role influencing responsibility to determine character influence larvae even in the nucleus and modification ecdysis effects that determine the process of change in the cuticle and the turn instar stage. The role of the hormone is then applied to the artificial hormone pyriproxyfen and methoprene. IGR is a hormone that is controlling the growth of biorational control, which is a process that affects the growth of larva-pupa to adult ratio by regulating hormonal biology itself larvae that affect physiology of morphogenesis in the larval-pupal stage that will shape the mosquito pupa and disabilities resulting from the furnishing inexcessive dosage [20].

Larvae that survive will grow into pupae, but there has been a maturation and cellular differentiation thus become abnormal growth pupa. Larvae undergo ecdysis, the replacement process is not complete because the cuticle skint hat should be separated from the body of the larvae turned out to cohere in the posterior, thus masking tool that works spiracle respiration, this result can not breathing pre-pupae and finally die. In the pupa stage, juvenile hormone does not work anymore, but the hormone active ecdyson serves to form organs, organs and organ systems respiration [21].

In general, IGR can give the effect of long-term survival (3 to 6 months) with an appropriate dose. This is consistent with research Pranoto that shows the percentage of deaths pupa until the fourteenth week amounted to 99.25%. So that repetition of administration can be done for 3 months [18].

CONCLUSIONS

Effective dose of Pyriproxyfen to kill 50 % of larvae of *Aedes aegypti* is 1.009 ppm, while to kill 90 % larvae is 1.704 ppm. Effective dose of Methoprene to kill 50 % of larvae of *Aedes aegypti* is 1.342 ppm and effectively kill 90 % at a dose of 2.704 ppm. Pyriproxyfen have better effectiveness in killing the larvae of *Aedes aegypti* compared with Methophren.

Further research needs to be done to determine the effectiveness pyriproxyfen and methophren in the field so that it can be applied directly in the community, and ultimately can help reduce morbidity and mortality due to dengue fever.

REFERENCES

1. Mansjoer A, 2001. Demam Dengue. Dalam Ilmu Penyakit Dalam Infeksi Trofik. Edisi 3 Jilid 1. Jakarta, Media Aesculapius,
2. Dinas Kesehatan Provinsi Kalimantan Selatan. 2012 Profil Kesehatan Provinsi Kalimantan Selatan Tahun 2011.
3. Krueger. 2006. Effective Control of Dengue Vectors With Curtains and Water Container Cover Treated With Insecticide in Mexico and Venezuela: Cluster Randomized Trial. *BMJ* 75: 1247-52
4. Zulhasril dan Frieda FB. Penggunaan piretroid sintetik (permetrin) dalam pengendalian larva vector demam berdarah dengue (Aedes aegypti L). J Kedokt Yarsi 2006;14(1): 29-33
5. Sungkar, S., Zulhasril. 1997. Status kerentanan larva *Aedes aegypti* terhadap temefos di beberapa daerah di Jakarta. *Majalah Kedokteran Indonesia* 47: 25-28.
6. de Carvalho, M.D.S., Caldas, E.D., Degallier, N., Vilarinhos, P.D.T., de Souza, L.,Amelia, M., Yoshizawa, C., Knox, M.B., de Oliveira, C. 2004. Susceptibility of *Aedes aegypti* larvae to the insecticide temephos in the Federal District, Brazil. *Revista De Saude Publica* 38: 623-629.
7. Rodriguez, M.M., Bisset, J., Ruiz, M., Soca, A. 2002. Cross-resistance to pyrethroid and organophosphorus insecticides induced by selection with temephos in *Aedes aegypti* (Diptera : Culicidae) from Cuba. *Journal of Medical Entomology* 39: 882-888.
8. Gafur A, Mahrina, Hardiansyah. 2006. Kerentanan larva Aedes aegypti dari Banjarmasin Utara. *Bioscientiae* 3(2) : 73-82
9. Istiana, Heriyani F, Isnaini. 2012. Status Kerentanan larva Aedes Aegypti terhadap Temephos di Banjarmasin Barat. Jurnal Buski 4(2) : 1-6.
10. WHO, CDS, WHOPES, GCDPP. 2005. Guidelines for Laboratory And field Testing of Mosquito Larvicides.
11. Rodriguez, M.M., Bisset, J., De Fernandez, D.M., Lauzan, L., Soca, A. 2001. Detection of insecticide resistance in *Aedes aegypti* (Diptera : Culicidae) from Cuba and Venezuela. *Journal of Medical Entomology* 38: 623-628.
12. Campos, J., Andrade, C.F.S. 2001. Larval susceptibility to chemical insecticides of two *Aedes aegypti* populations. *Revista De Saude Publica* 35: 232-236.
13. Andrighetti MTM, Cerone F, Rigueti M, Galvani KC, da-Gracia-Macoris MdL. 2008. Effect of pyriproxyfen in *Aedes aegypti* populations with different levels of susceptibility to the organophosphate temephos. Dengue Bulletin 32 : 186-198.

14. Munif A. Pengaruh residu pyriproksifen 0,5% terhadap pertumbuhan larva aedes aegypti pada berbagai simulasi wadah air formula tepung pada berbagai instar larva Aedes aegypti di laboratorium. CDK 1997;119(2) : 42-9
15. Braga IA, Mello CB, Peixoto AA, Valle D. Evaluation of methoprene effect on Aedes aegypti (Diptera : Cullicidae) development in laboratory conditions. Mem Inst Oswaldo Cruz, Rio de Janeiro 2005: 100(4) : 435-40
16. Wu Y, Parthasaraty R, Bai H, Palli SR. Mechanisme of midgets remodelling : juvenile hormone analog methoprene block midgut methamorphosis by modulating ecdysone action. Mechanisme of development 2006;123(7) : 537 – 40
17. Braga IA, et al.2005. Effectiveness of methoprene, an insect growth regulator, agains temephos resistant Aedes aegypti populations from different Braziliense localities, under laboratory condition. J Med Entomol 42: 830-837
18. Pranoto. 1994. Pengaruh Insect Growth Regulator (IGR) Altosid 1z,3G terhadap populasi Larva nyamuk Aedes aegypti linneus. Buletin Penelitian Kesehatan 1994;22(4):1-9
19. Maharani A, Handayani FD. Insect growth regulator (IGR) terhadap larva Aedes aegypti di laboratorium. Profesi Medika 2005;5(2):67-75
20. Kamal HA, Khater El. 2010. The biological effect of insect growth regulators : pyriproxyfen and diflubenzuron on the mosquito Aedes aegypti. J Egypt Soc Parasitol.40(3):565-74
21. Ponlawat, A., Scott, J.G., Harrington, L.C. 2005. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus*across Thailand. *Journal of Medical Entomology* 42: 821-825.

THE EFFECTIVENESS OF PERMETHRIN AND METHOPRENE AS LARVICIDES TO *Aedes aegypti* LARVAE FROM WEST BANJARMASIN

Nelly Al Audhah
Departement of Parasitology, Medical Faculty of Lambung Mangkurat University
Correspondence: audhah_fkunlam@yahoo.co.id

ABSTRACT

Background: The resistance to temephos is widely identified. Alternative use of larvicides such as permethrin and metophrene for combating the spread of dengue fever has not been studied in Banjarmasin making necessary studies to determine its effectiveness. This study aimed to determine the effectiveness of permethrin and metoprene as larvicides and concentration required to kill 50 % of larvae of *Aedes aegypti* from Banjarmasin West district.
Method: This was an experimental study with posttest -only control group design. Permethrin dose was 0.0015, 0.0018, 0.002, 0.0022, 0.0025 and 0.0030 g/l, while methopren 0.0, 0.0063, 0.0025, 0.005, 0.01 and 0.1 g/l respectively. Each treatment group was exposed to *Aedes aegypti* larvae. Larval metamorphosis and mortality were followed for 10 days. Data was analyzed by independent t test and probit.
Results: The results showed that the effective dose of permethrin to kill 50 % of larvae (Lethal Concentration/LC_{50}) for 24 hours ranged from 0.001439 to 0.001829 g/l, with an average of 0.001671 g/l. While 24 hour LC_{99} ranged from 0.002856 to 0.003852 g/l with an average of 0.003196 g/l. This study showed that the larvae of *Aedes aegypti* in West Banjarmasin districts are resistant to permethrin. As for the larvicidal, active ingredient of methopren kill 50 % of larvae on the seventh day in dose of 0.1 g/l.
Conclusion: This study showed that the use of larvicides IGR group can be an alternative in areas that have been declared as resistant to temephos in the district of West Banjarmasin.
Keywords: effectiveness, permethrin, methophrene, *Aedes aegypti*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is an infectious disease that becomes a public health problem, especially in tropical developing countries. The incidence of DHF in Indonesia in 2007 reached 139.695 cases (incidence rate / IR 64 cases per 100,000 populations) with the number of patients who died reaching 1,395 cases (case fatality rate / CFR 1 %). South Kalimantan Provincial Health Department (2007) reports that dengue cases in 2008 occurred in all districts with IR 16.73 cases per 100,000 populations and the most mortality is in Banjarmasin for 6 people. Along 2012, dengue cases in the province of South Kalimantan reached 1216 cases with the number of patients who died more than 19 cases (case fatality rate / CFR 1.5 %). This number increased three times compared to 2011 that was only 400 cases with 7 deaths. Banjarmasin city is the third most dengue cases with 56 people. However, mortality case reached 3 people with CFR 5.3 % exceeding provinces CFR [1].

Dengue controlis currently based on the eradication of the vector which is *Aedes aegypti*. *Aedes aegypti* eradication can be done by eradicate adult mosquitoes and larvae through Abatization. However, some studies indicate resistance of Aedesa egypti's larva toward abates, based on research by Gafur et al. [2] and Istiana et al. [3] who stated that in Banjarmasin North and West resistance has occurred.

World Health Organization (WHO) has recommended the use of permethrin for dengue fever eradication program as an alternative to dengue endemic areas who have used temephos in the long term such as in Banjarmasin [1]. Besides,WHO also recommends the use of Insect Growth Regulator (IGR) such as methophren.The objectiveof this study is to determine the effectiveness of permethrine and pyriproksifen larvicides against *Aedes aegypti* larvae in the district of West Banjarmasin. This study is expected to provide scientific information as well as the basis for policy implementation control for growth and spread of mosquitoes causing dengue fever in endemic areas.

METHOD

This study is an experimental research with Posttest-Only Control Group Design. The subjects were larvae of *Aedes aegypti*instar III-IV of second generation that have been colonized in Parasitology Laboratory, Medical Faculty, Lambung Mangkurat University, Banjarmasin. The independent variable in this study is the dose/concentration of permethrin which is 0.0015 g/l, 0.0018 g/l, 0.0020 g/l, 0.0022 g/l, 0.0025 g/l, 0.0030 g/l; methopren with concentrations of 0g/l, 0.0065 g/l, 0.0125 g/l, 0.025 g/l, 0.05 g/l and 0.1 g/l, the dependent variable is the mortality of larvae of *Aedes aegypti* after administration of larvicides. The method in this study is in line with the method set by the WHO for experiments in the laboratory and the obtained data were analyzed using probit through statistical computer.

RESULTS

Table.1 Average mortality of Aedes aegypti larva in the Western District of Banjarmasin after administration ofpermethrin1%

Replication	Larvae	Permetrin 1% dose						
		0 g/l	0,0015 g/l	0,0018 g/l	0,0020 g/l	0,0022 g/l	0,0025 g/l	0,0030 g/l
	number of larvae	25	25	25	25	25	25	25
1	Number of dead	0	11	13	21	24	24	24
	% mortality	0	44	52	84	96	96	96
	number of larvae	25	25	25	25	25	25	25
2	Number of dead	0	12	13	13	22	25	25
	% mortality	0	48	52	52	88	100	100
	number of larvae	25	25	25	25	25	25	25
3	Number of dead	0	9	16	22	12	24	24
	% mortality	0	36	64	88	48	96	96
	number of larvae	25	25	25	25	25	25	25
4	Number of dead	0	10	11	11	24	25	20
	% mortality	0	40	44	44	96	100	80
average % of mortality		0	42	53	67	82	98	93

Observed data of *Aedes aegypti* larvae mortality after administration of permethrin at various concentrations in 24 hours can be seen in Table 1.It showed that the percentage of deaths in the control group was 0%, which means that the death of larvae was only affected by the larvicides administration. At the lowest dose (0.0015g/l) result in the death was an average of 42%. Along withincreasing doses, the larval mortality percentage is also higher with 100% mortality occurred at doses of 0.0025g/l. Table 2 showed the calculation of the dose probit analysis toward Permethrin against *Aedes aegypti* larvae for 24 hours.

Table 2. Probit analysis calculation result of permethrin dose

Larvae mortality (%) in 24 hours	**pure permetrin dose (g/l)**
10	0.00083035
20	0.00111879
30	0.00132678
40	0.00150450
50	**0.00167060**
60	0.00183670
70	0.00201442
80	0.00222241
90	0.00251085
95	0.00274905
99	**0.00319588**

The results using larvicides that were included in the IGR, which was methoprene, can be seen in Table 3 below. Observations on the larvae of the test and control was conducted for 10 days with aims to determine the length of time needed to reach test larval mortality for more than 50 %.

Table 3. Percentage of larval mortality after Methophren exposure for 10 days

Days	Percentageof larval mortalitywith consentration **(%)**					
	0g/l (control)	**0,0065 g/l**	**0,0125 g/l**	**0,025 g/l**	**0,05 g/l**	**0,1 g/l**
1	0	0	0	0	0	0
2	0	0	1,25	1,25	1,25	1,25
3	0	3,75	1,25	1,25	7,5	6,25
4	2,5	7,5	11,25	3,75	8,75	17,5
5	2,5	10	17,5	26,25	25.5	32.25
6	2,5	11,25	25	30	37.75	46.25
7	3,75	22,5	35	33,75	55.75	65
8	6,25	51,25	55	57,5	60	80
9	7,25	67.5	70.0	70.0	71.5	84.25
10	8.5	70.0	72.25	74.75	74.75	90.0

DISCUSSION

Probit analysis results of permethrinis appraised LC50 24 hours 0.001439-0.001829 g/l, with an average 0.001671 g/l and 24-hour LC99 ranged between 0.002856-0.003852 g/l with an average of 0.003196 g/l. This result is very much different from the study by Zulhasril et al [4], which the LC50 and LC95 probit analysis

is 0.000257 g/l and 0.000667 g /l. The differences are caused by different strains of test larva so then the response was also not the same.

Permethrin in Indonesia is mostly used as adulticide for eradication of dengue vectors. Formulation is still available in the form of Emulsion Concentrate (EC), but its importance as larvicides are still in early stages of research in the laboratory. Currently there is no application in the field as larvicidesthat are usually provided in the form of granules.The unavailability of permethrin formulation in granule form because there is insufficient research report on the effectiveness of permethrin as larvicides and the suspicion of cross resistance among chemical insecticides [5].

Table 3 showed that in the control group, the test larval mortality until day 10 was 8.5 %. It meant that the death was not caused by the treatment of other factor except metophrene administration. It was seen on day1or after exposure larvicides for 24hours, the average test larval mortality was 0 %, or there has been no death. On day 2 was seen death although still in very small number. The longer and the higher the dose given, the percentage of death is also increasing.The observation in Table 3 also showed that at day 7, only the 0.05 g/l and 0.1 g/l dose groups which gives more than 50% mortality. While on the last day of observation, day 10, the average percentage of larval test mortality for all doses is more than 70 % and the highest concentrations reached 90 % percentage of mortality.

Increased mortality along with the length of time due to the IGR metophrene active substance did notkill larvae directly, but inhibits the growth that can lead to death in the pupa stage and growth failure of at adult mosquito's stage [6]. This is according to research which is conducted by Braga et. al. [7] stated that the greatest mortality percentage on test larvae which is given methoprenis in pupa stage, not in the larval stage [4]. The same thing also expressed by Wu et al [8], that the increasing concentration of methopren, the percentage pupa mortality is also increased. According Pranoto [9], percentage pupa mortality reached 100 % in the third week after treatment. It can support the results of this study that indicate methopren inhibit the development of larvae and kills the pupa stage. Because on the fifth day, in the control group, there were a lot of mosquitoes mature and grow up on the tenth day. However, none of the treatment groups of larvae mature to adult mosquitoes up to tenth days.

On larvae with methopren exposure, proliferation and differentiation of imaginal cells is initiated during 36 hours on 4 thin star larvae, but the cell death program is initiated only during the pupal stage. As a result, the development of pupae with methopren exposure contains two layers of epithelial on midguts until they died during the pupal stage. Thus, the methopren interfere re-establishment midguts that were modulated by the expression of several genes involvedin 20E signal transduction [7].

Kruskal Wallis test analysis results $p= 0.240$, which means there is no significant difference of percentage of test larval mortality in all groups. To determine significant differences among treatment groups, the Mann-Whitney test is done. The results is indicate that there are several groups of $p<0.05$. This means that there are significant differences in some treatment groups which is among the control group with the group with a concentrationof 0.05g/l and 0.1g/l.

In general, IGR can survive in long-term (3 to 6months) with an appropriate dose. It is in line with Pranoto's research that shows the percentage of pupa survival's deaths up to fourteenth week become 99.25%. So that repetition of administration can be done for 3months [9].

CONCLUSION

Effective concentrations of permethrin to kill 50% of larvae within 24 hours of the test is 0.001671 g/l and 99% effective in killing larvae is 0.003196 g/l. Methopren effective dose to kill 50% of larvae of Aedes aegypti was 0.05 g/l and 0.1 g/l on the seventh day, and the effective dose to kill 70% of larvae was 0.065 g/l, 0.0125 g/l, 0.025 g/l; 0:05 g/l and 0.1 g/l on day 10. Further research needs to be done to determine the effective dose of Methophren and Permethrin that can be used by the publicas larvicides against Aedes aegypti.

REFERENCES

1. Dinas Kesehatan (DoH) Provinsi Kalimantan Selatan. 2007. Profil Kesehatan Provinsi Kalimantan Selatan Tahun 2006.
2. Gafur A, Mahrina, Hardiansyah. 2006. Kerentanan larva *Aedes aegypti* dari Banjarmasin Utara. *Bioscientiae* 3(2) : 73-82
3. Istiana, Heriyani F, Isnaini. 2012. *Status Kerentanan larva AedesAegypti terhadap Temephos di Banjarmasin Barat*. Jurnal Buski 4(2) : 1-6.
4. Zulhasril dan Frieda FB. Penggunaan piretroid sintetik (permetrin) dalam pengendalian larva vector demam berdarah dengue (Aedes aegypti L). J Kedokt Yarsi 2006;14(1): 29-33
5. Suwasono H, Damar TB, Hasan B, Mujiyono, Raharjo. Efikaasi permetrin dengan aplikasi ULV terhadap Culex quenquefasciatus. SDK 2001:13:1
6. Maharani A, Handayani FD. Insect growth regulator (IGR) terhadap larva Aedes aegypti di laboratorium. Profesi Medika 2005;5(2):67-75
7. Braga IA, Mello CB, Peixoto AA, Valle D. Evaluation of methoprene effect on Aedes aegypti (Diptera : Cullicidae) development in laboratory conditions. Mem Inst Oswaldo Cruz, Rio de Janeiro 2005: 100(4) : 435-40
8. Wu Y, Parthasaraty R, Bai H, Palli SR. Mechanisme of midgets remodelling : juvenile hormone analog methoprene block midgut methamorphosis by modulating ecdysone action. Mechanisme of development 2006;123(7) : 537 – 40
9. Pranoto. Pengaruh Insect Growth Regulator (IGR) Altosid 1z,3G terhadap populasi Larva nyamuk Aedes aegypti linneus. Buletin Penelitian Kesehatan 1994;22(4):1-9

THE RELATIONSHIP BETWEEN FOGGING TIME AND FREQUENCY WITH THE INCIDENCE OF DENGUE HEMORRHAGIC FEVER (DHF) AT GRIBIG HEALTH CENTER, MALANG 2008-2009

Lilik Zuhriyah[1], Intan Yusuf Habibie[2], Aswin Djoko Baskoro[3]
[1]Department of Public Health, Faculty of Medicine, Brawijaya University
[2]Study Program of Nutrition, Faculty of Medicine, Brawijaya University
[3]Department of Parasitology, Faculty of Medicine, Brawiijaya University
Correspondence: lilikz.fk@ub.ac.id

ABSTRACT

Background: Dengue hemorrhagic fever (DHF) is an infectious disease that often leads to outbreaks and accompanied by high mortality rate. The number of DHF cases at Gribig Health Center showed an increase between 2008-2010, which was 67, 126, and 166 cases respectively. Fogging remains regarded as a means of primary prevention of DHF by the community. There are many factors affect the implementation of fogging, including the time and frequency of fogging. This research aimed to find out the relationship of fogging time and frequency with incidence DHF at Gribig Health Center.
Methods: This was an observational study with ecological approach. The study was conducted on January-March 2011. Study population was villages in Kedung Kandang Subdistrict. Sampels were Lesanpura, Madyopuro and Sawojajar. Independent variables were fogging time and fogging frequency, while dependent variable was incidence rate of DHF. Data of 2008-2009 was obtained from secondary data drawn from Gribig Health Center. Then, the data was analyzed by spearman correlation test because of its abnormal data distribution.
Results: The results showed that fogging time dan fogging frequency significantly positively related to the incidence of DHF ($p<0.0001$; $r=0.432$ and $p=0.002$; $r=0.372$ respectively). Yet the increase of fogging frequency could not decrease the incidence rate of DHF. In addition, the time of fogging implementation at Gribig Health Center did not suit the guideline given by Ministry of Health 2005.
Conclusion: Fogging could not reduce the incidence of DHF.
Keywords: fogging, time, frequency, incidence, DHF

BACKGROUND

Dengue Haemorrhagic Fever (DHF), which can manifest as Dengue Shock Syndrome (DSS) is an deadly infectious disease. Mode of transmission is through mosquito vectors *Aedes aegypti* and *Aedes albopictus*. This endemic disease, often cause outbreak and high mortality [1]. In recent decades, the incidence of DHF showed increased very rapidly throughout the world. A total of two and a half billion, or two-fifths of the world's population is at risk of dengue fever. A total of 1.6 billion (52 %) of the population who are at risk live in the Southeast Asian region. Since located in the tropical zone of the equator, dengue fever is still a major problem in Indonesia, Myanmar, Sri Lanka, Thailand and Timor-Leste. All Indonesia area are at risk for contracting the disease because the dengue virus and its vectors are widespread, both in

the home and public places, except for the area which its altitude is more than 1,000 feet above sea level [2]. During the years 2003-2007, the number of dengue cases showed a significant increase . the number of cases from 2003 until 2007 were 51.516 cases , 79.462 cases, 95.279 cases, 114.656 cases, and 158.115 cases [3].

East Java is one of the provinces mostly affected by dengue fever cases after Jakarta and West Java. The incidence of dengue fever in East Java increased during the years 2004-2007, i.e. 8.287 cases, 14.796 cases, 18.484 cases and 24.186 cases respectively, while deaths cases were 120 people, 264 people, 217 people, and 340 people respectively. From January to September 2010 the cases recorded were 22.040 with 190 deaths. From 38 districts/cities, there were 10 dengue endemic areas are, i.e. Pacitan, Ponorogo, Tulungagung, Madiun, Kediri, Malang, Surabaya, Probolinggo, and Situbondo District [4]. Number of dengue cases in Malang in 2008, 2009, and 2010 are 408 cases, 614 cases, and 879 cases respectively with number of deaths is 3, 4, and 5. Kedungkandang subdistrict is an area with the second highest number of dengue patients in Malang city, where the number of dengue cases in 2008-2010 were 67, 126, and 166 cases respectively [5].

There are three factors that play a role in the transmission of dengue infection, namely humans, viruses, and vectors. Dengue virus enters body when mosquitoes bite people who are experiencing viremia, and dengue virus is transmitted to humans through the bite of infectious *Aedes aegypti* and *Aedes albopictus*[6]. Until now vector control remains the best option to reduce the number of DHF patients Vector control activities can be done by eradication of mosquito breeding places, epidemiological investigation program, selective abatization, fogging, and public health promotion [7].

Fogging is still regarded as the primary means of prevention by people. Fogging is only recommended for vector control in emergency situations to suppress the ongoing epidemic or to prevent the emergence of a new case. Fogging is aimed to reduce adult mosquito quickly. However, until now there is still controversy regarding the effectiveness of aerosol insecticide applications during the epidemic. Many methods for reducing the number of adult mosquitoes are infective, even in a short time, but it is still not clear whether this temporary significant impact in the long time. There has been no evidence of the effectiveness of this method in dealing with cases of epidemic. However, if spraying is done at the beginning of the epidemic and in a large enough scale, intensity of transmission can be reduced. Not only insecticide susceptibility, the droplet dose, frequency of spraying, implementation time, and insecticide spreadinginto rooms also have vital effects to the success of fogging in controlling *Aedes aegypti*[8]. This study aimed to analyze relationship between fogging time and frequency with the incidence of DHF in Gribig Health Center, Malang in 2008-2009.

METHODS

Research design

This was an ecological study.

Population and sample

Population was entire village located in Malang city. Sample consisted of Lesanpuro, Madyopuro, and Sawojajar. These villages are in working area of Gribig Health Center.

Location and time
The study was conducted in Gribig Health Center including Lesanpuro, Madyopuro, and Sawojajarvillages of Kedungkandang subdistric, Malang City, in January-March 2011.

Instrument and data collection
This study used secondary data from Gribig Health Centerand village office of Lesanpuro, Madyopuro, and Sawojajar in Kedungkandang Subdistrict, Malang to determine fogging frequency, fogging time and the incidence of dengue.

Independent variables
Fogging timewas defined as date and month of fogging implementation before the month of DHF incidence, whichwas done by Health Office and/or independent parties in 3 villages per month. Fogging frequency was the number of fogging activities carried out by Health Office and/or independent partiesin 3 villages permonth. Data of fogging frequency before and current month of incidence of dengue was taken from Puseksmas Gribig 2008-2009.

Dependent variable
Incidence of was defined DHF asnumber of DHF patients per number of population in each villages per month. We assumed number of population was stabile each year. Data of DHF incidence was based on records from Gribig Health Center 2008-2009.

Data analysis
Kolmogorov-Smirnov test showed data were not normally distributed so the data was analyzed using Spearman correlation test to determine the relationship between the fogging time and fogging frequency with incidence of DHF.

RESULTS

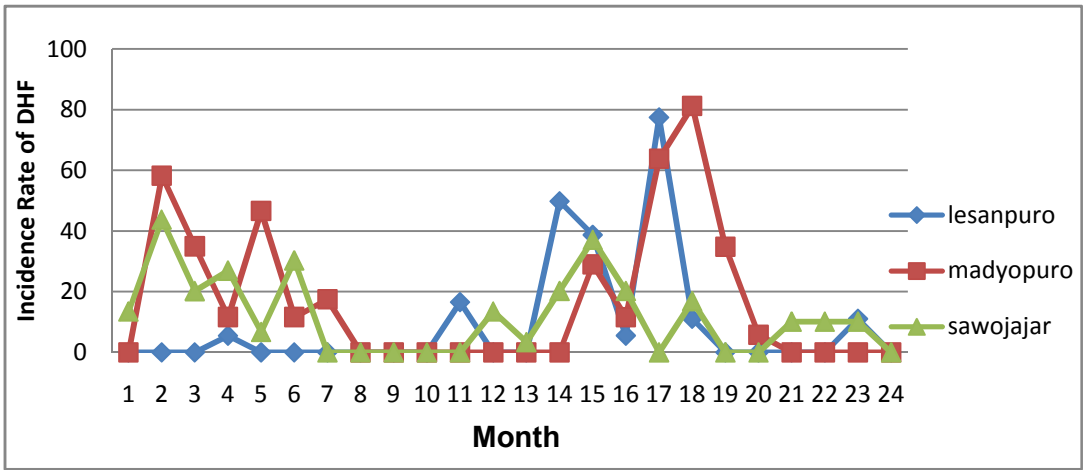


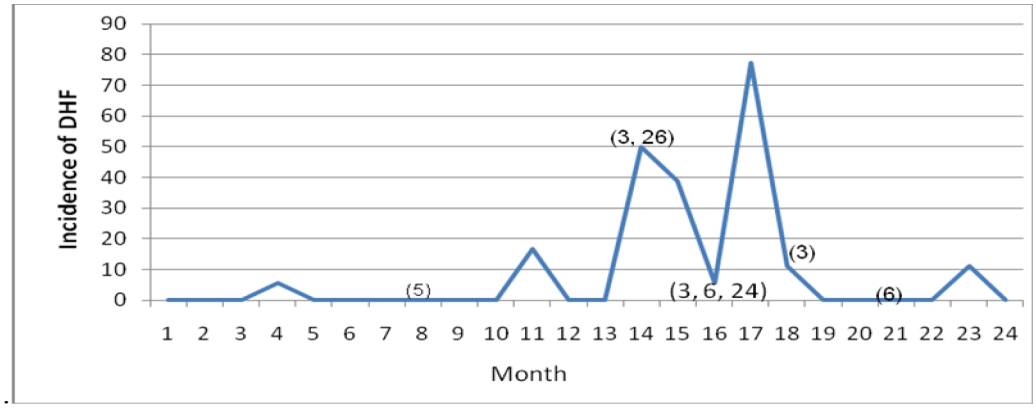
Figure 1. Incidence rate of DHF at working area of Gribig Health Center, Kedungkandang Subdistrict, Malang City, 2008-2009

Based on data from the village offices in 2008 and 2009, population of Lesanpuro, Madyopuro, and Sawojajar village were 18,124 and 18,071, 17,172 and 17,243, and 29.711 and 29.651 people respectively. Dengue incidences in 2008 and 2009 in Lesanpuro were 22.06 per 100,000 and 193.65 per 100,000 population, in Madyopuro were 180.5 per 100,000 and 226.14 per 100,000 population, while in Sawojajar village were 154.8 per 100,000 and 128.11 per 100,000 population. By assuming that population number was constant over 1 year, monthly data of Incidence Rate are shown by Figure 1.

Based on data from Gribig Health Center, average of fogging frequency in 2008-2009 in Lesanpuro was 4 times each year with the lowest fogging frequency 0 and highest times 3 times per month. In Madyopuro, the average fogging frequency was 5 times each year with the lowest fogging frequency 0 and highest times 2 times every month. In Sawojajar, average fogging frequency was 7.5 (8 times) every year with the lowest fogging frequency 0 and highest times 3 times per month

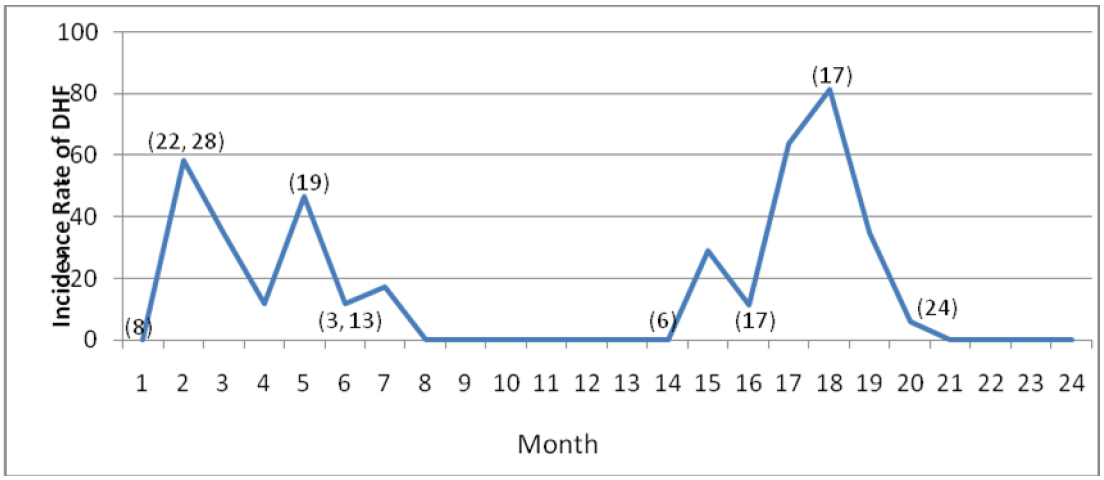
Tabel 1. Fogging time, fogging frequency, rainfall, and number of rainy days in working area of Gribig Health Center, Kedungkandang Sub District 2008-2009

Month	2008			2009		
	Fogging Frequency	Rainfall (mm)	Number of Rainy Days	Fogging Frequency	Rainfall (mm)	Number of Rainy Days
January	2	253	13	1	343	17
February	3	164	15	7	424	16
March	1	372	24	1	281	12
April	0	94	5	4	154	11
May	1	38	4	0	203	7
June	3	0	0	2	0	0
July	0	0	0	0	0	0
August	2	0	0	3	0	0
September	0	0	0	2	0	0
October	0	103	3	0	0	0
November	0	257	17	0	0	0
December	1	269	9	0	0	0



Note : : Incidence of DHF ; (...) : Date of *Fogging time*

Figure 2. Fogging time and incidence of DHF at Lesanpuro Village 2008-2009



Note : : Incidence of DHF ; (...) : Date of *Fogging time*

Figure 3. Fogging time and incidence of DHF in Madyopuro Village 2008-2009

Relationship between fogging frequency one month before and dengue incidence rate

Spearman correlation test indicated there was an average relationship between fogging frequency one month before and dengue incidence in the Gribig Health Center (r=0.432, p=0.002). Based on Figure 4, it can be conclude that fogging frequency one more before did not reduce the incidence of dengue.

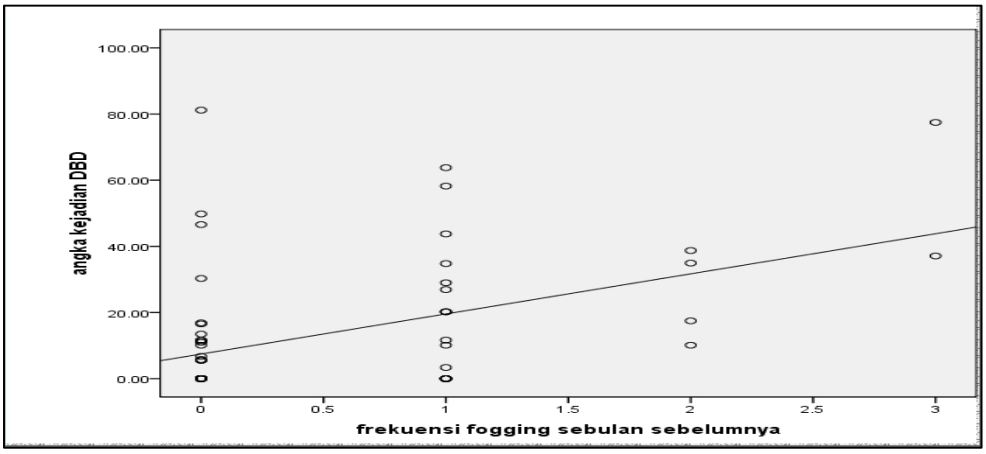


Figure 4. Relationship between fogging frequency one month before and incidence rate of DHF with positive direction

Relationship between fogging time and incidence rate of dengue

Spearman correlation test between one month before fogging time and the incidence of DHF showed there was an average relationship between the frequency of the power being fogging during the incident with the incidence of dengue in the Gribig Health Center (r=0.372, p<0.0001). Figure 5 showed increased fogging time did not reduce the incidence of dengue.

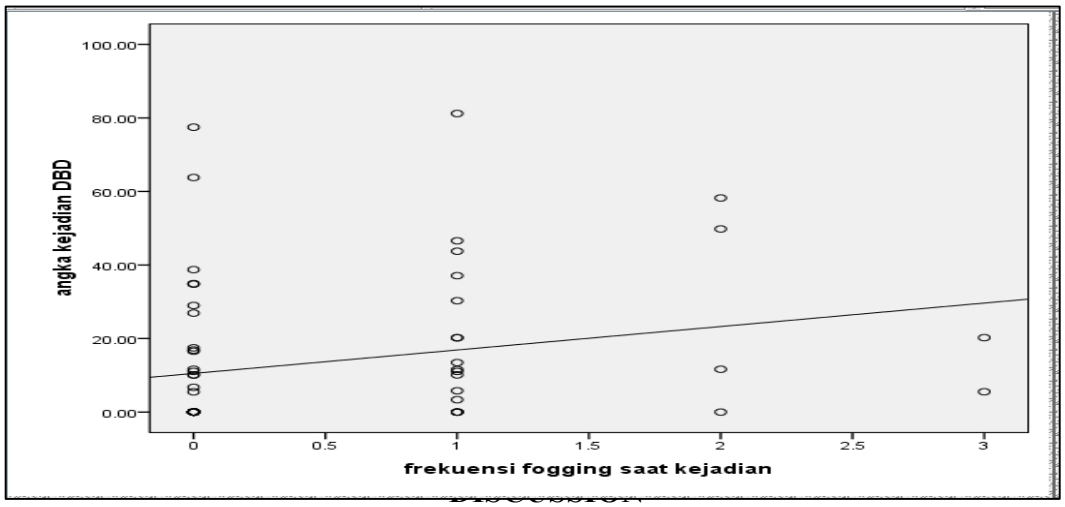


Figure 5. Relationship between fogging frequencywith incidence rate dengue with positive direction

DISCUSSION

Relationship between fogging time with dengue incidence rate

Table 1 indicated there is incompliance in the fogging time as advised by the MOH in 2005. In guidelines, it is explained that in order to limit the transmission of dengue virus, spraying should be conducted two cycles with intervals of one week. In the first cycle of spraying, all mosquitoes containing dengue virus and other mosquito will die. But soon after that, new mosquitoes will appear to suck blood patients and can cause re-infection. Therefore, it is necessary to spray the second cycle. The second spraying is conducted one week after the first spraying in order to eliminate and prevent the infective mosquitoes infect people. In an emergency situation with a high incidence of DHF, ideally fogging should be done every 2-3 days for 10 days. The next, it is done once or twice a week to maintain the adult vector population suppression. Epidemiological entomology and vector must continue to be done to determine the exact schedule of spraying and effective control strategies [9].

The results also showed that timing of fogging in Kedungkandang Sub District did not consider rainfall. Though WHO has stated that the implementation of fogging was also affected by weather conditions, including rainfall [9]. There are two kinds of fogging, namely mass and focus foggings. Mass fogging iscarried out before and after rainy season in DHF high risk area. Meanwhile, focus fogging can be done in the rainy season when dengue cases are found. This is because the fogging contain malathion substances that are toxic and harmful to the children and under five years old [10].

The mismatches in fogging time might be causedby lack of fogging effectiveness in reducing the incidence of dengue in this area so that the incidence of DHF is still high. This result is similar with Fathi et al [11], who stated that it did not seem the role of action spraying (fogging) on DHF outbreaks in Mataram (Chi-square, $p>0.05$). In contrast, fogging provides a false sense of security to people who can disrupt mosquito breeding cleanup program like '3 M ' and abatization.

Relationship between fogging frequency andincidence rate dengue

There are manyfactors influincing high incidence of dengue fogging instead of high fogging frequency.At the time of the DHF outbreak, frequent fogging with insecticides malathion in home environment was intended for adult mosquitoes. If fogging activities are not supported by the application abatization, then in a few days will rise again the density of adult mosquito because of larvae are not killed by fumigation will be mature.Therefore the eradication ofvector in adult stage (fogging) should be accompanied by abatization [12]. WHO in 2009 [9] also explained that indoor spraying is recommended only for vector control in emergency situations to suppress the ongoing epidemic or to prevent the emergence of a new case. This is supported by our resultthat fogging frequency, which is conducted in the incident, is more effective than fogging frequency one month before the incident.

Many factors affect the implementation of fogging. Not only the frequency of fogging, but also the vulnerability of insecticide and droplet dose. The entry of insecticide into the room also affects vital to the success of this method for controlling *Aedes aegypti*. The entry of insecticide into the room dependon the structure of the building, if doors and windows are left open during spraying, fogging time, the housing block configuration, route of the spray, and weather conditions. Several other factors must also be considered in the spraying, they are safety, wind speed, vector, biological effectiveness, the availability of equipment,space operations, and personnel capabilities. The things that need to be considered in the implementation of fogging to get optimal results are: a) Concentration of the solution and how to make it. For malathion, the concentration of the solution is 4-5 %, b) Nozzle must be used in accordance with the solvents used and the desired output discharge, c) Distance snout engine with a maximum target of 100m, or 50m for effectiveness, d) Walking speed when fogging, for fog swing approximately 500 m2 or 2-3 minutes for the home and yard, e) Fogging timeshould be adjusted to density/peaktime of the mosquito activity, ie 09.00-11:00 [13].

Gede Suarta *et al* [14] stated that execution of fogging focus in the fight against dengue disease has not been optimized so that dengue cases are still high. This was due to some technical implementation focus fogging is still not in accordance with the guidelines or technical guidance. Human resource officers and supervisory personnel fogging has not received training on the implementation of technical, causing fogging focus becomes less effective. Delay reports received from health facilities cause epidemiological investigation and enforcement activities fogging focus becomes too late. It was also the cause of the lack of effective implementation of fogging focus. In addition, the monitoring function performed health workers, village officer have not been going well so some practice is not in accordance with the guidance.

As was explained earlier that the implementation of fogging was also influenced by the droplet and the dose of chemicals used. Currently many emerging phenomenon of resistance to insecticides commonly used materials include: malathion, temephos, tenthion, permethrin, profoxur, and fenithrothion.Based on data from the Provincial Health Office in Jakarta in 2008, the insecticides used for fogging in the area of Jakarta is malathion that has been mass used since 1969. Besides, PHO Jakarta also used the temephos, which is larvicidal to mass kill larvae *Ae.aegypti* that has been used since 1980. Malathion and temefos contain organophosphate active ingredients. The use of these insecticides for long periods can cause *Ae. aegypti* resistance to the active

ingredient. It is because of in fogging not all *Ae. aegypti* was killed, mosquitoes still alive by escape from insecticides or insecticide doses insufficient contact with mosquitoes. Consequently, mosquitoes are becoming resistant and the resistance was inherited to the offspring. There have been several studies that support the existence of such resistance. *Ae. aegypti* larvae in Yogyakarta likely resistant to malathion and temefos. Research carried out the Department of Parasitology in collaboration with Government of DKI Jakarta in 2007, reported mostly in Tanjung Priok *Ae.aegypti* larvae which were resistant to organophosphate insecticides were 44.8 % and 50 % were resistant to very resistant. In Mampang Prapatan, most larvae *Ae. aegypti* also been resistant to the organophosphate insecticide were 57.2 % in moderate level and 9.8 % in high level [15]. Several factors have been mentioned here are probably less noticed by the community and local health authorities, causing a lack of effective implementation of fogging in reducing the incidence of dengue in the Gribig Health Center, Kedungkandang Subdistrict.

Limitation of study

Limitation of this study is the use of secondary datafor data collection, ie fogging frequency, fogging time, and the incidence of dengue. There is a possibility of error in recording the data. In addition, jumantik (larvae examiner) and local health authorities may not record the fogging time on a regular basis so that fogging in the urban village of incoming data to the clinic or local health service is not complete.In addition, the use of ecological studies in this research also lead to limitation, because ecological studies cannot be used to analyze the causal relationship because of the inability to bridge the gap exposure status and disease status at the population level and the individual, and not able to control for potential confoundingfactors.

CONCLUSION

Based on the research and analysis of data, it can be concluded that the fogging time is significantly positively associated with the incidence of dengue in the Gribig Health Center Kedungkandang Sub District. Implementation fogging can not reduce significantly the incidence of dengue.Fogging frequency has significant positive assicuatuib with the incidence of dengue in the Gribig Health Center. Increasing fogging frequency was not able to reduce the incidence of dengue.

Further research should focus on other factors that affect the implementation of fogging, such as dosage, the spraying, wind speed, and weather conditions to determine the relationship with the incidence of dengue. Health care workers need to pay attention to the implementation of guidelines and educate the public about the fogging implementation, especially the need for two-cycle fogging. There needs to be a better recording system for the fogging time, fogging frequency and incidence of DHF in each village.

REFERENCES

1. Indrawan, 2001. Mengenal dan Mencegah Demam Berdarah. Bandung: Pioner Jaya.
2. Depkes RI Ditjen Pengendalian Penyakit Dan Penyehatan Lingkungan. 2007. Pencegahan Dan Pemberantasan Demam Berdarah Dengue Di Indonesia. Jakarta.

3. Depkes RI. 2003. Indonesia Sehat 2010. Jakarta: Depkes.
4. Ica. 2010. Jatim Potensi KLB DBD, (www.harianbhirawa.co.id, diakses 15 Desember 2010).
5. Dinkes Kota Malang. 2010. Data Penderita Demam Berdarah Kota Malang Tahun 2008-2010. Malang: Dinkes.
6. Depkes RI Ditjen Pengendalian Penyakit Dan Penyehatan Lingkungan. 1992. Nyamuk Penular Demam Berdarah Dengue. Jakarta: Depkes.
7. Depkes RI Ditjen Pengendalian Penyakit Dan Penyehatan Lingkungan. 1992. Pemeriksaan Kuman Penyakit Menular. Jakarta: Depkes.
8. WHO. 1997. Dengue Guidelines For Diagnosis Treatment Prevention And Control. Geneva.
9. WHO. 2009. Dengue Guidelines For Diagnosis Treatment Prevention And Control. Geneva.
10. Dinkes Kota Pekanbaru. 2010. Antisipasi DBD-3M Lebih Baik dari Fogging (www.pekanbaru.go.id, diakses 7 Januari 2012)
11. Fathi, Keman, Soedjajadi., Wahyuni, Chatarina Umbul. 2005. Peran Faktor Lingkungan dan Perilaku terhadap Penularan DBD di Kota Mataram. Jurnal Kesehatan Lingkungan, Vol. 2, No.1, Juli 2005: 1-10.
12. Depkes RI Ditjen Pengendalian Penyakit Dan Penyehatan Lingkungan. 2005. Pencegahan Dan Pemberantasan Demam Berdarah Dengue Di Indonesia. Jakarta.
13. Iskandar A, dkk. 1985. Pedoman Bidang Studi Pemberantasan Serangga dan Binatang Pengganggu APTKS. Jakarta: Pusdiknakes Depkes RI.
14. Gede S., Retna S., Lutfan L. 2009. Evaluasi Pelaksanaan Fogging dalam Penanggulangan Demam Berdarah Dengue di Kota Denpasar. Thesis. UGM. Jogjakarta.
15. Felix. 2008. Demam Berdarah Dengue, Sebuah Polemik yang Harus Dituntaskan. (www.majalah-farmacia.com, diakses 15 Februari 2012)

THE CHANGES OF CHOLINESTERASE ACTIVITIES AND ELECTROLYTE (NA,K) SERUM BEFORE AND AFTER THE SPRAY ON RED ONION SPRAYER FARMER IN SUKOMORO NGANJUK

Hansen
STIKes Insan Cendekia Medika Jombang
Correspondence: hansenskm@yahoo.co.id

ABSTRACT

Background: Sukomoro subdistrict take place in Nganjuk district, East Java is the centre of red onion producer. The preliminary survey in Sukomoro subdistrict, showed that the spraying of pesticide in red onion farm was done three to four times in a week, starting from planting until a week before harvesting. At certain months, the spraying was done intensively, once a day, to avoid pests. Pesticides are hazardous materials that can cause negative impacts on human health and environmental sustainability. This study is aimed at making analysis of the altered activities of cholinesterase activities, sodium and potassium in a serum before and after the spraying on red onion farmers in Sukomoro subdistrict.
Method: This was an analytic observational study with cross sectional approach. The study was conducted on March-June 2013. Data retrieval was perfomed in May-June 2013. Study subject consisted of 17 participants. They were observed before and after the spraying activity The data collection was done through questionnaires and the examination of the level of cholinesterase and electrolyte serum. Data was analyzed using paired t-test and linear regression.
Results: The study showed that there was significant difference between sodium ($p=0.037$) and potassium ($=<0.0001$) levels before and after sprating, Linear regression indicated a relationship between wearing protective equipment ($p=0.001$), length of exposure ($p=0.019$) and cholinesterase.
Conclution: Rhere was a significant difference between sodium and potassium before and after the spray, the existence of a meaningful relationship between wearing of protective equipment, length of exposure and cholinesterase.
Keywords: electrolyte, sodium, potassium, cholinesterase, pesticide

BACKGROUND

The agricultural activities in Indonesia nowadays are predominantly agroindustry-oriented in which the use of pesticide as pest control is ubiquitous both in farming and plantation [1]. It has been known that some types of pesticides, especially organochlorine, can stays on soil for years, while water resources in the area become susceptible to contamination due to excessive use of pesticide, accidental application of pesticide or external application in the river or in the pool in order to control the growth and spread of weeds [2].

Most cases of accidental pesticide poisoning have been reported to happen among the farmers and their respective families, the exposure of which takes place during the mixing and applying stages. An acute exposure also may take place during its

preparation, formulation, packaging, and distribution. The acute effect as a result of pesticide exposure takes various forms ranging from eye burn sensation, skin damage, until neurological one. Cronical exposure, on the other hand, is oftenly linked to reproductive harm, cancer risk, neurological and psychological effects, heart and liver problems besides immunity issue [3].

Aside from its harmful effect on human health, pesticide accounts for enviromental hazard. Pesticide rate in the enviornment shall rise when it stays in the environment, or when biomagnifications tends to occur. With regard to its endurance in the environment [4], pesticide falls into nonpersistent pesticide (less residue) where its persistence rate range from 1-12 weeks in soil and water media; almost persistent (medium residue) with time to breakdown ranges from 1-18 months; and persistent pesticide (high level residue) with persistence rate of 2-5 years [5].

The purpose of this research is to analyze the change in the activity of cholinesterase, electrolyte (sodium and potassium) in blood serum before and after the pesticide application among onion growers in the sub-district of Sukomoro, Nganjuk Regency.

METHOD

This was an analytic observational study using cross sectional approach, the location of the research is sub-district of Sukomoro in Nganjuk Regency. Date of research started in March and ran until June 2013 while data compilation started in Mei and ended in June 2013. Population was all onion growers in onion fields in subdistrict of Sukomoro, Nganjuk Regency. Sample was the onion growers in the onion field organized in a farmer group in the farming area located in sub-district of Sukomoro in Nganjuk Regency. A total of 17 respondents were analyzed before and after the applying of the pesticide done, all of which were selected using simple random sampling technique.

As dependent variables were the cholinesterase activity in blood serum as well as electrolyte (sodium and potassium) of the growers pre and post applying, while the independent variables included pesticide dose response, protective equipment use, hours of exposure, spray frequency. The data collected herein are of primary ones with direct observation of each variable. Secondary data used herein are obtained from geographical data of sub-district office, community health center, and regency agriculture office.

This research uses paired-samples t-test for analyzing the change in cholinestarase, sodium and potassium prior to the spray and afterward. Linear regression test is employed to analyze the correlation among the variables in the test.

RESULT

Based on paired-samples t-test shown in table 1 it is safe to conclude that there is no significant change of pre- and post applying, in contrast to sodium and potassium which evidently shows significant change. Table 2 shows that risk factor related with cholinesterase level among onion growers in sub-district of Sukomoro, Nganjuk Regency is the most highly exposed ($p=0.019$; $\beta=-0.462$), so that the longer respondents exposure to pesticide the lower their cholinesterase level, with the highest

cholinesterase risk occurs among respondents who use improper protective equipment (p=0.001; β=0.390).

Table 1 Mean levels of the cholinesterase, sodium and potassium before and after the pesticide application on onion growers in the subdistrict of Sukomoro, Nganjuk 2013.

Variable	Differences				Change pre-post applying pesticide		p
	Pre		Post				
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Sodium	140,76	3,527	142,47	1,940	-1,706	3,098	0,037
Potassium	4,200	0,4272	3,200	0,2937	1,0000	0,5635	0,000

Table 2 Linear regression analysis on several factors related with cholinestarase, sodium and potassium among the onion growers in Sub-District of Sukomoro, Nganjuk 2013.

Variable	Cholinesterase (post)	
	β	p
High Exposure	-0,462	0,019
Personel Protective Equipment	0,390	0,001

DISCUSSION

Cholinesterase level in blood samples taken from respondents after a short break from spraying may rise to some extent (table 1). The finding is in contrast to another study on workers of pesticide formulation at PT. Bina Guna Kimia Klepu Ungaran that recorded a change in cholinesterase activity before and after their work done (p<0.0001) [6], due to long exposure, approximately 8 hours/day, to pesticide, where the amount of pesticide sucked in became greater. Another study confirms that the discrepency in cholinesterase level before and after applying pesticide returns to baseline level after two week break [7].

The research result concerning sodium level in blood serum before and after the application of pesticide using paired samples t-test shows a significant rise, not with standing its normal change, reveals that some amount of pesticide has been absorbed by the kidneys through inhalation, skin as well as gastrointestinal system during eating or drinking right after applying pesticide causing it to build up in larger quantities in the kidneys leading to nephrotoxicity and harms the kidneys, especially the tubules, which is susceptible to hazardous chemicals or drugs [8,9]. A study on goldfish and rat exposed to organophosphate pesticides has confirmed such as damage on proximal tubules. Hispathology of the kidneys was observed under a microscope after pesticide injected in the kidneys of the goldfish and of the rat [8,9].

The significant change, a decline, in potassium level in blood serum before and after the applying of the pesticide using paired-samples t-test reveals that pesticide deposited in the kidneys builds up so that decreases potassium level in blood serum which in turn can damage the tubules, the reason why electrolyte test confirms the changes in both sodium and potassium levels. The previous experiment cited on the goldfish and the rat has evidently confirmed the conclusion [8-10].

Table 2 shows a correlation between hours of exposure and the decline in cholinesterase level in the blood serum of onion growers. It is safe to say that the longer the exposure, the lesser the cholinesterase level. It happens due to excessive applying of pesticide, namely more than five hours/day so that the poison absorbed by the body is higher than that among respondents who work $\leq$ 5 hours/day. The result is in line with another study confirming that respondents who are exposed to pesticide > 5 hours/day are more risky of pesticide poisoning compared to those who are exposed $\leq$ 5 hours/day [2] due to the higher amount of the pesticide sucked in by the body. Another study finds that fertile women with high exposure level to pesticide are 2.5 times more susceptible to declining of cholinesterase level compared to fertile women with low exposure to pesticide, not to mention the fertile women working, mostly clearing weed, with no protective equipment on, while their husbands apply the pesticide, the risk of exposure is evidently higher compared to fertile women with protective equipment on [11].

The analysis result shows that there is a correlation between the improper use of protective equipment and pesticide poisoning in the body, in this case is the rise in cholinesterase level in blood serum among onion growers, most probably due to $\pm$ 30 minute-break after the applying of the pesticide prior to blood sampling. Another factor involved is the pesticide mixture where the higher its concentration the faster the decline of cholinesterase level and vice versa [12]. The proper use of protective equipment can reduce direct exposure of pesticide toward the body of the appliers so that reduce the risk of pesticide poisoning which occur through inhalation, swallowing, or skin contact [13].The result is in contrast with another study who shows that protective equipment use is linked to pesticide poisoning in blood serum considering the improper use of protective equipment among the respondents in the study [14]. As stated earlier, improper use of protective equipment among pesticide sprayers is more susceptible to harmful exposure and poisoning compared to proper use of protective equipment. Another study confirms that pesticide appliers with improper use of protective equipment face the risk of poisoning 5.9 times higher compared to those with proper use of protective equipment. The reason is obvious, the improper use of protective equipment allows pesticide exposure to happen in high degree, and vice versa [15].

REFERENCES

1. Departemen Kesehatan., *Pengenalan dan Penatalaksanaan Keracunan Pestisida*, Subdit Pengamanan Pestisida, Jakarta, 1992, Hal 5-21
2. Tugiyo., Keracunan Pestisida pada Tenaga Kerja Perusahaan Pengendalian Hama di DKI Jakarta *Tesis, 2003*
3. WHO, *Hazardous Chemicals In Human and Environmental Health*, EGC, Jakarta, 2000.
4. Lu FC., *Basic Toxicology : Fundamental, Target Organs, and Risk Assesment*, Hemisphere Publishing Corporation, New York, 1995
5. Mukono, *Toksikologi Lingkungan*, Airlangga University Press, Surabaya, 2010
6. Kartikarini., Perbedaan Aktifitas Kolinesterase Sebelum dan Sesudah Bekerja (Penelitian Pada Tenaga Kerja Formulasi Pestisida di PT. Bina Guna Kimia Klepu Ungaran, *skripsi,*Fakultas Kesehatan Masyarakat, Universitas Diponegoro, 2002

7. Raini M., Dwiprahasto I., Sukasediati N., Pengaruh Istirahat terhadap Aktivitas Kolinesterase Petani Penyemprot Pestisida Organofosfat di Kecamatan Pacet Jawa Barat., *Bul Penelitian Kesehatan*, Vol 32 No. 3, 2004 : 105 – 111
8. Kerem M., Bedirli N., Gorboz N., Ekinci O., Bedirli A., Taylan., Sakrak O., Pasaoglu H., Effects of Acute Fenthion Toxicity on Liver and Kidney Function and Histology in Rats, *Turk J Med Sci*, 2007; 37 (5): 281-288.
9. Singh RN., Histopathological Alteration In The Kidney of Cyprinus carpio After Exposure To Dimethoate (EC 30%), *Indian J. Sci. Res* 3(1) 2012 : 127 – 131
10. Baldatina AZ.,Pengaruh Pemberian Insektisida (Esbiothrin, Imiprothrin dan D-Phenothrin) Pada Tikus Putih (Rattus rattus), Kajian Histopatologi Hati dan Ginjal, *Skripsi,* Fakultas Kedokteran Hewan, Institut Pertanian Bogor, 2008
11. Purba IG., Analisis Faktor-Faktor Yang Berhubungan Dengan Kadar Kolinesterase Pada Perempuan Usia Subur Di Daerah Pertanian, *Tesis,* Program Pascasarjana Universitas Diponegoro Semarang, 2009
12. Gossel T.A., *Principle of Clinical Toxicology.* 2nd Ed. Raven Press, New York, 1990
13. Djojosumarto P., *Pestisida dan Aplikasinya*. PT. Agromedia Pustaka. Jakarta, 2008
14. Afriyanto, Nurjazuli, Budiyono., Keracunan Pestisida Pada Petani Penyemprot Cabe Di Desa Candi Kecamatan Bendungan Kabupaten Semarang., *Jurnal Kesehatan Lingkungan*, 2009, Vol 8 No. 1
15. Yuantari MGC., Studi Ekonomi Lingkungan Penggunaan Pestisida Dan Dampaknya Pada Kesehatan Petani di Area Pertanian Hortikultura Desa Sumberrejo Kecamatan Ngablak Kabupaten Magelang Jawa Tengah, *Tesis,* Program Pasca Sarjana Universitas Diponegoro Semarang, 2009 Cyprinus carpio After Exposure To Dimethoate (EC 30%), *Indian J. Sci. Res* 3(1) 2012 : 127 – 131

TOXICITY OF CHIP (MAT) WASTE OF ELECTRICAL MOSQUITO REPELLENT TO RESPIRATION ACTIVITY OF SOIL MICROORGANISMS

Iva Rustanti, Irwan Sulistio.
School of Environmental Health, Poltekkes Kemenkes Surabaya
Correspondence: ivarust.eri@gmail.com

ABSTRACT

Background: Electric mosquito repellent chip or mat is widely used in community. One of its uses a combination of pesticides d-allethrin and transfluthrin. Gas Chromatography test results showed that mat-waste still contain residual d-allethrin-transfluthrin, so the waste supposed to be included in the B3. The purpose of this study was to analyze the toxicity of mat-waste on respiration activity of soil microorganisms.
Method: This was an eco toxicological test for the toxicity effects of mat-waste on soil microorganisms. The concentration of treatment was 1, 3, 5, and 7g (per kg soil) for 3, 5, 7, 10, 15 and 20 days of exposure. The data were analyzed using log probit.
Results: The greater concentration of mat-waste presented increasingly longer exposure time, giving greater toxicity effects, and decreases the concentration of CO_2 produced by respiration of soil microorganisms. Value of non observed effect concentration (NOEC) waste mat on soil microorganisms could not be determined because the log probit model was U-shaped (following the model E).
Conclusion: Mat-wastewas toxic but not lethal or only slightly toxic effect soil microorganisms.
Key words: roxicity, electric mosquito repellent chip (mat), soil microorganisms

BACKGROUND

Source of soil contamination was basically derived from any activity that could result a waste which could enter to the ground then contaminated it [1]. One of material contaminants was pesticides both in the domestic and agricultural environments [2]. One form of pesticide applications which usually used by people was in a piece or fragment form of electric mosquito repellent or vaporizing mat (called mat) and it was quite safe. The instructions usage listed on the product packaging explained that the mat was already used up must be replaced with new ones after using it within ± 8 hours. Used mat products had been known containing the active pesticide compound, which consists of d-alletrine, transflutrine, metoflutine, sipenotrine, with varying concentrations [3].

Packaging product did not include mat instructions of handling after usage, resulting in people dumped the used mat into domestic garbage and did not separate it out from other domestic wastes. mat-user surveys conducted by Avani [3], 70.71% people of Surabaya whom used electric mosquito repellent, threw the waste directly to the domestic garbage, and only 29.29% of consumers whom collected the waste into a special container before it was trashed.

Soil microorganisms are composed by mostly of bacteria, fungi and microalgae [4]. Soil microorganisms played a role in the mineral cycle, nitrogen fixation, revamp pesticide residues, humification process, fertilize the soil, reform the hazardous waste, biodegradation (the breakdown of organic matter by microbial activity which involved a series of enzymatic reactions, used as a food source), bioremediation (the use of organisms to clean up polluting substances from the environment), mineralization, decomposition, and others. Populations of soil microorganisms are influenced by the amount and various nutrients in the soil, moisture, oxygen, temperature, and pH [5].

People's habit by throwing mat-waste, which remain contain active ingredient of pesticide, may contaminated soil. Therefore, a research or study was required to examine the waste of toxic effects (toxicity) in electric mosquito repellent fragment toward the respiration activity of soil microorganisms.

METHOD

Material

The test soil used in this study was a garden soil with pH 6.5 and temperature of 28^{o}C. Larger number of soil microorganism was $1x10^{4}$ colonies/kg soil. Electric mosquito repellent fragment (mat) used in this study contained pesticide compounds of d-alletrin and transflutrin. As stated in the product packaging, the concentration of d-alletrin and transflutrin in these fragments, respectively for 40 mg and 3 mg. Mat-waste derived from used mats which were applied for 8 hours continuously.

Soil respiraton test

This stage was performed to determine the volume of CO_2 respiration activity of soil microorganisms. Soil Respiration Test was presented in figure 1. Soil respiration activity was observed by the indicator of CO_2 onset on result microorganisms in soil respiration. The procedure of these observations was to mix the soil with various concentrations of mat-waste. Water and aerated was added in each mixture about 10 minutes to provide an aerobic condition. The result of soil microorganisms'CO_2 respiration activity would come out through the air pipe (hose) in the reactor. The volume of CO_2 was measured by looking at the high aerial of the calibrated beaker. Observations were made during 10 days of exposure.

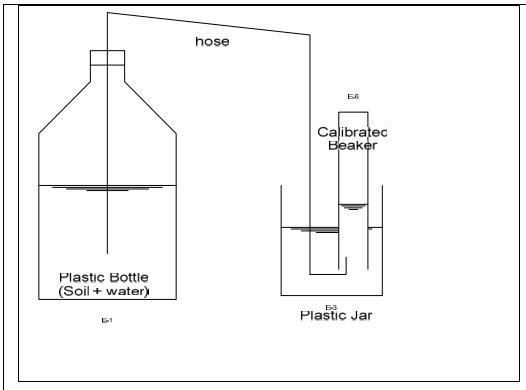


Figure 1. Soil respiration test reactor

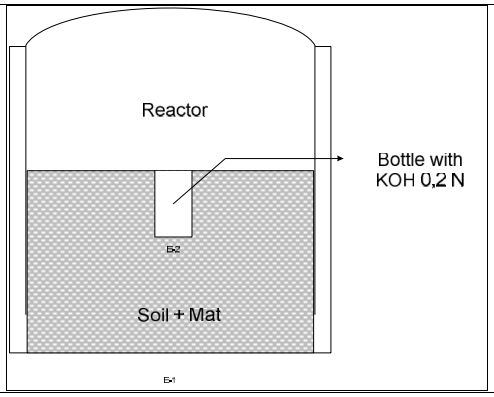


Figure 2. Mat toxicity reactor test towards soil microorganisms respiration

Mat-waste toxicity test on soil microbial respiration activity

Mat-waste toxicity test was a stage to determine the level of mat-waste toxicity towards CO_2 respiration activity of soil microorganisms result, with the variety of themat-waste concentration at 1 ppm mat, 3 ppm, 5 ppm and 7 ppm with exposure time for 3, 5, 7, 10, 15, and 20 days respectively. The equipment used in this phase was the toxicity test reactor (Figure 2) and the materials needed were electric mosquito repellent fragments (mat) which had been heated for 8 hours, a solution of KOH 0.2 N, 0.2 N HCl solution, indicator solution phenolpthalein and methyl orange indicator.

Mat-waste toxicity was observed by CO_2 onset gas indicator of respiration result in soil microorganisms which were bounded by KOH 0.2 N solution. The procedure of these observations was to mix the soil test and mat-waste with a determined concentration, and was exposed for 3, 5, 7, 10, 15 and 20 days. CO_2 result of respiration activity of soil microorganisms captured by 0.2 N KOH solution, which was put in the middle of small bottle in each test reactor. To prevent CO_2 respiration activity of soil microorganisms went out to the air, thus, the reactor was covered with plastic. At the end of each period, KOH solution in a small bottle was titrated with 0.2 N HCl solution that were added phenolpthalein indicator previously. Test result analysis used formula as follow:

$$[\ CO_2\ ]_{\text{respiration output}} = \frac{(\text{vol HCl 1} - \text{vol HCl 2}) \times N\ \text{HCl} \times 10^{-3} Td}{\text{(obtained result in mol/day)}}$$

Vol HCl 1 = Titrated Volume HCl control (ml); Vol HCl 2 = Titrated Volume HCl blanko (ml); N HCl = 0.2 N; Td = days of exposure

RESULT

Table 1. Gas chromatography analysis resultof waste mat and new mat

Chemical Material	existence of material	
	Mat (New)	Mat (waste)
d-allethrin or (RS)-3- allyl-2- methyl-4-oxocyclopent-2-enyl (1R)-cis,trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	+	+
Transflutrin or (1R-trans) - (2,3,5,6-tetrafluorophenyl)-methylester 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylic acid	+	+
Solvent		
1) 2,4,6-pyrimidinetrione,5-ethyl-5-(3-methylbutyl)-5-ethyl-5-isopentyl-2,4,6-pyrimidin	+	+
2) Benzenamine,2,6-dichloro-4-nitro, 1-amino-2,6-dichloro-4-nitrobenzene,2,6-dichloro-4-nitroamine	+	+

CO_2 volume (ml) soil respiration result of soil microorganisms

Soil Respiration Test was conducted to determine the volume of CO_2 respiration activity of soil microorganisms. Soil Respiration Test measurement method used capillary method as practiced by Avani [3]. Our result showed the greater reduction of water, the more CO_2 respiration results released by soil microorganisms. Soil Respiration Test was carried out for 7 days could be seen in Figure 3.

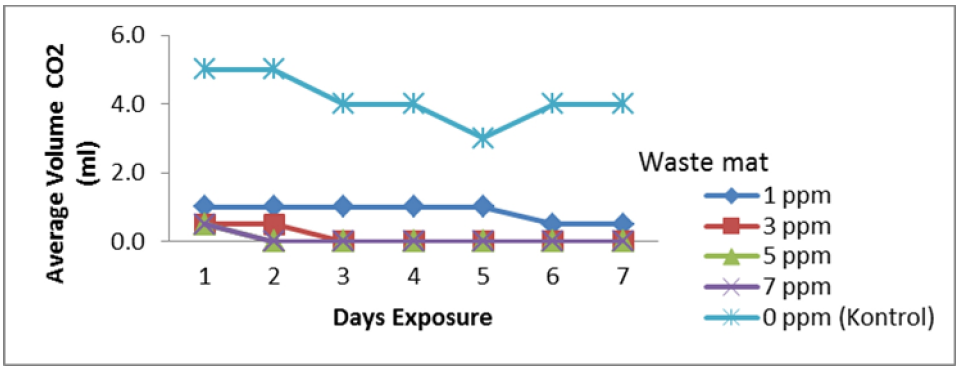


Figure 3. Average Volume of CO_2 (ml) Soil RespirationResults of Soil Microorganisms

Measurement result in Figure 3 showed that in water, mat-waste was quite toxic to microorganisms. For mat-waste with a concentration of 1 ppm/250 ml soil solution still had its respiration activity from day 1 to 7 with an average volume of 0.86 ml CO_2. Mat-waste with a concentration of 3 ppm/250 ml soil respiration activity was only until day 2 with an average CO_2 volume of 0.5 ml, on day 3 to 7 its respiration activity was undetectable. On mat-waste exposure concentration of 5 ppm and 7 ppm, respiration activity was detected only on day 1 with CO_2 volume of 0.5 ml, for day 2 to day 7, while respiration activity of microorganisms was not detected. The average volume of CO_2 results respiration activity of microorganisms in the condition without mat-waste exposure was 4.14 ml. According to Mangkoedihardjo [6], the toxic effect as expressed with the term *Toxicity Unit (TU)*, a substance exposure could be measured by the volume of gas transforming activity of microorganisms as a result of massive exposure effect concentration (EC).

$$\mathbf{TU = [(1/EC)_i – (1/EC)_0]/ (1/EC)_i \times 100\%}$$

Toxicity Value Unit > 50% meant a substance included in the category of high toxic. From the equation above, it could be obtained the score of Toxicity Unit for each exposure concentration mat-waste as presented in Table 2.

Table 2.Waste-mattoxicity exposure unit at concentration of 1 g, 3 g, 5 g, 7 g (per250 ml soil solution) on Soil Respiration Test

Mat concentration (gr/250ml lar.soil)	Average Volume CO_2 (ml)	EC (ml)	Toxicity Unit (%)
1	0,86	0,86	79,31
3	0,5	0,5	87,93
5	0,5	0,5	87,93
7	0,5	0,5	87,93

EC_0 = volume $CO_2$of non-exposure microorganism respiration result = 4.14 ml

Table 2 showed that the mat-waste Toxicity Unit on soil respiration test was more than 50%, therefore, the category of mat-waste was considered as high toxic [6].

Mat-waste toxicity test on soil microbial respiration activity

Toxicity test was conducted to determine the toxic effects of pesticide residue remaining in the waste of electric mosquito repellent fragment (mat) on the respiration activity of soil microorganisms during the exposure within 20 days, which was calculated by the amount of CO_2 produced from respiration of soil microorganisms that were captured by 30 ml solution of KOH 0.2 N. Furthermore, in the KOH titrated solution with HCl 0.2 N.

$$\mathbf{2\ KOH\ + CO_2 \rightarrow K_2CO_3\ + H_2O.....(2)}$$

$$\mathbf{KOH\ + HCl \Leftrightarrow KCl + H_2O.....(3)}$$

Figure 4 showed that at each mat-waste exposure concentration and control, the longer the exposure time, the greater the amount of CO_2 respiration result, it showed that there had been accumulation of CO_2 respiration result captured by KOH 0.2 N solution. While the average of CO_2 (mg) result in the respiration control of soil microorganisms showed a higher rate than the average of CO_2 soil respiration which was exposed by mat-waste.

The amount of microorganisms in soil test after mat-waste exposure

The amount of microorganisms in the soil test after waste-matexposure was measured by the examination of Total Plate Count (TPC). Figure 5 described that at each exposure concentration with long exposure of matin 3, 5, 7, 10, 15 and 20 days, the amount of soil microorganism colonies were still bigger than the minimum number of colonies for normal soil ($1x10^{-4}$ colonies / kg soil). The result of amount of microorganism colonies could not demonstrate the influence of exposure significantly towards changes in the number of mat-waste microorganism colonies.

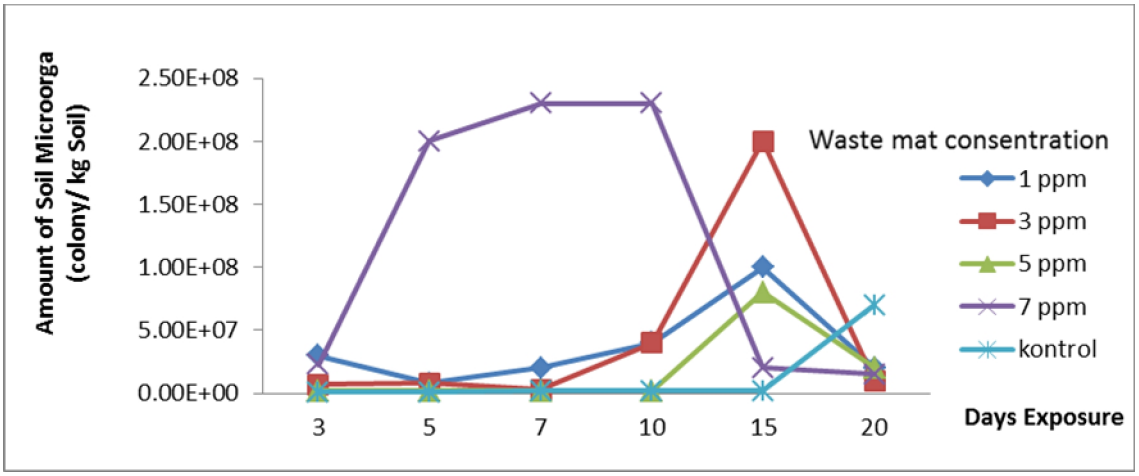


Figure 5. Amount of soil microorganisms (colony / kg soil) after waste-mat exposure at 1 ppm, 3 ppm, 5 ppm, 7 ppm and exposure control in 3, 5, 7, 10, 15 and 20 days

Log Probit Model

To see the correlation between the mat-waste exposure concentration with effects on the environment in the form of CO_2 result in a soil microorganism used respiration

Log Probit model was showed in Figure 6. Log probit model was presented in the figure above explained the non-linear form (not a straight line), but shaped like the letter U.

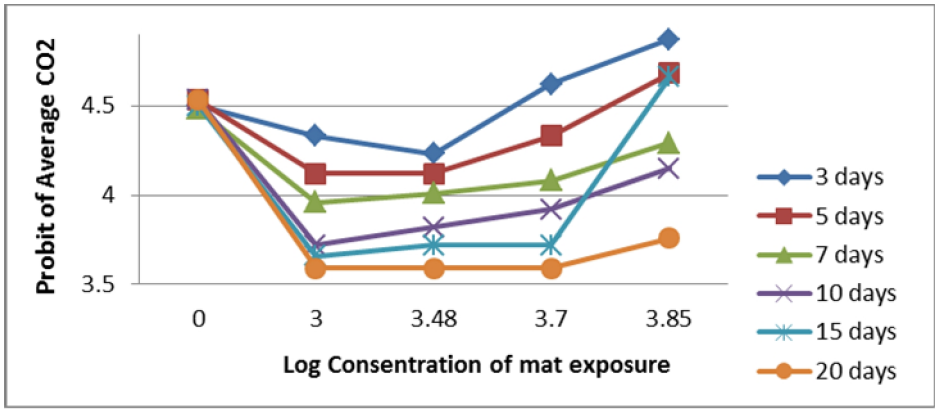


Figure 6. Log Probit model with CO2 concentration Waste-mat Respiration Microorganisms exposure time per day in 3 days, 5 days, 7 days, 10 days, 15 days and 20 days

DISCUSSION

Figure 5 described that the exposure of mat waste not demonstrate influence significantly changes in the amount of soil microorganism colonies According to Trihadiningrum [7], several soil microorganisms had cell organelles that could form spores in its cell. Organelles known as endospores could protect against exposure to soil microorganism pesticides or other toxic compounds. If the environmental condition was possible to grow, there would be a sporulation, where cell degeneration dam endospores would be released. This provides information that if a particular soil microorganism experienced acute condition or chronic toxicity because of the exposure to a toxicant, the other types of soil microorganisms would advantage from its position as the new environmental condition.

According to Buikema Jr.et al, 1982 in Mangkoedihadjo [6], log probit model like the letter U occurred was because the test material was toxic but did not give effect to toxic or just gave a little toxic effect on the target organisms. Qualitative test resulted d-alletrin content of pesticides and waste transflutrin on mat in Table 1 indicated that the solvents used as a solvent d-alletrin and transflutrin were lipophilic or more easily dispersed in fat than water, with a score of POW (octanol partition - water) was quite high at 4.95 [8]. POW high score also indicated that the bond between the solvent with the active ingredients of pesticide was very strong and stable, making it relatively difficult to disperse in the soil. Furthermore, because of the lipophilic nature, it would slow down the contact between the reactions of pesticide with microorganisms.

This condition informed that the pesticide in waste-mat was not easily released into the soil environment, especially if the soil in dry condition. However, in wet soil or in the water, pesticide in the mat-waste was easier to be dispersed because of the diffusion mechanism occurred as the principle fugasiti substances. Fugasiti of d-alletrin and transflutrin were in liquid form, thus, if these two active substances were in the aquatic environment, they would tend to be dispersed into the aquatic environment [3].

It is fitted with the measurement result of the waste-mat exposure effect on the amount of CO_2 respiration of microorganisms result in the Soil Respiration Test (Figure 3). Mat-waste soil respiration exposure test was conducted on soil solution; hence, it had a significant result on the influence of mat-waste exposure to the large volume of CO_2 respiration microorganisms result, with more than 50% of Toxicity Unit.

CONCLUSION

Mat-waste exposure concentration with the longer time of exposure would provide greater toxicity effects, which decreased the concentration of CO2 produced by respiration of soil microorganisms. Non Observed Effect Concentration value of waste-matexposure to soil microorganisms could not be determined because the log probit model was in U-shaped form, which meant that the material test (mat- waste) had toxic characteristic but did not give toxic effects or just gave a little toxic effect on the target organisms (soil microorganisms).

REFERENCES

1. Siregar, I., Hermana J., 2001. Kajian Pengaruh Respirasi Mikroorganisme Tanah Tercemar Cadmium dengan Penambahan Larutan Basa ($Ca(OH)_2$). Jurnal Purifikasi. Vol.2. No.3. ITS. Surabaya
2. Connel . D.W, Miller. G. J, 1995. Kimia dan Ekotoksikologi Pencemaran. Univercity Indonesia Press. Jakarta.
3. Avani, Endah, 2008. Kajian Ekotoksikologi Keping Anti Nyamuk Elektrik BekasPakai Terhadap Mikroorganisme Tanah dan Biota Air. Surabaya : ITS
4. Soil Conservation Service US Department of Agriculture, 1993. Soil Survey Manual Handbook 18.
5. Jaya, B.S. Hermana, J. 2001. Uji Pengaruh Toksisitas Logam Berat Cadmium (Cd) dan Usaha Detoksifikasi Logam Berat Seng (Zn) pada Tanah. Jurnal Purifikasi. Vol.2. No.5. ITS. Surabaya.
6. Mangkoedihardjo, S. 2009. Ekotoksikologi Teknosfer. Guna Widya. Surabaya
7. Trihadiningrum Y, 1995. Mikrobiologi Lingkungan. Jurusan Teknik Lingkungan – ITS Surabaya
8. WHO, 2002. Specification and Evaluation for Public Health Pesticides: d-alletrine, Geneva.

SPATIAL DISTRIBUTION AND BREEDING CHARACTERIZATION OF *Anopheles sp*. IN SELAYAR ISLAND, SULAWESI, INDONESIA

Hasanuddin Ishak[1], Amirullah[1], Isra Wahid[2]

[1]Postgraduate School of Public Health, Hasanudin University
[2]Dept. of Environmental Health, Faculty of Public Health, Hasanudin University
[3]Dept. of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
Correspondence: hasanuddin.ishak@gmail.com

ABSTRACT

Background: Malaria remains a major public health problem in Selayar Islands, Indonesia. This study aims to know the spatial distribution and breeding characterizations of Anopheles spp.
Method: This was a cross sectional study, conducted in Selayar Islands villages. Spatial data were analyzed using ArcView GIS. The characterization data were analyzed using SPSS Statistics 17.0 (Kruskall Wallis, Mann Whitney and Spearman Correlation).
Results: There are four *Anopheles spp* in Selayar islands village, *An. vagus*, *An. subpictus*, *An. indefinitus*, and *An. barbirostris*. Those species found in swamps, wells and water canal, while river and ponds was not breeding places. Breeding habitat characterizations effected the density of *Anopheles spp* larvae, i.e. habitat type (p=0.009), habitat width (p=0.004), water depth (p<0.0001), water clarity (p=0.002), water color (p=0.004), habitat sea level (p=0.03) and habitat shade (p=0.03). Whereas the existence of habitat (p=0.254) and water flows (p=07) has no effect larvae density.
Conclusion: It can be concluded that the distribution *An. subpictus*, *An. barbirostris*, *An. vagus*, and *An. indefinitus* depend on their habitat characterization.
Keywords: *Anopheles sp.*, spatial distribution, habitat characterization

BACKGROUND

Malaria is a major parasitic infection in the world, especially in tropical and subtropical countries (104 countries or 40% of the world population). Based on the data in the world, malaria kills one child every 30 seconds. Malaria is spread in about 100 poor countries in tropical and subtropical regions such as India, South America (except Chile), Afghanistan, Sri Lanka, Thailand, Indonesia, Vietnam, Cambodia, China, the Philippines, Central America, Mexico, and Africa [1]. A total of 107 million of Indonesia's population lives in malaria endemic areas, they are scattered in 310 districts in 32 provinces (about 70.3 %). In Indonesia, malaria spread throughout the island with the degree of endemicity different and can be popped up in the area with an altitude up to 1800 meters above sea level [2].

In South Sulawesi, the number of clinical malaria has increased from year to year, in 2010 there were 26,384 cases with a positive number by 4,547 cases. The highest cases was in the districts of Bulukumba, Selayar, North Luwu and Enrekang. Annual Parasite Incidence (API) in Selayar Islands in 2010 was 3.5 ‰ with 9 high case

incidence (HCI) villages, 15 moderate case incidence (MCI) villages, and 51 low case incidence (LCI) villages. In 2011, API (from January-May) was 2.03 ‰, while MDG 's target in 2015 is of < 1 ‰ [3].

Malaria prevalence remained occur in the Selayar islands from year to year due to several factors, among others, not all health centers have trained malaria control personnel, change management employee, officer mutation, and minimum financing for malaria control of the area. In addition, the main problem is also faced by many potential breeding habitat for malaria vectors such as lagoon, fish pond, a former quarry sand, marsh, pond, rainfed/irrigated non-technical, sand covered the mouth of the river, as well as the poor environmental sanitation [3].

Anopheles spp as malaria vector requires breeding sites that have characteristics consistent with the species. Cailly *et al*. [4] concluded that the larval and adult mosquito of *Anopheles melanoon* and *Culex modestus* respectively have a breeding habitat at irrigated fields and gardens that live by the reeds. Whereas *Anopheles arabiensis* has breeding habitat characteristics at three different geographical zones of North - East, West and South, among others, in the pool, river banks, and marshes [5]. Hardiman [6] showed on the spatial patterns of malaria mosquito breeding areas obtained results that mosquito breeding habitat has a tendency or trend in low-lying areas and urban areas. On the other hand, rainfall has a significant effect on mosquito density and the incidence of malaria on the island Kapoposang Pangkep [7].

Malaria elimination program in Indonesia stipulated in the Decree of the Minister of Health of Indonesia No. . 293/MENKES/SK/IV/2009 ,which basic vector data is need it . Data about the relationship between certain species in the environment is an important key in epidemiology [8]. According Adrial [9], one of the efforts to be made in the malaria eradication program is by eradicating the *Anopheles* mosquito larvae and species that act as malaria vectors. This effort was conducted in order to break the chain of transmission which in turn can reduce malaria morbidity in certain areas , because in the absence of epidemiological data concerning vectors , malaria eradication efforts will not succeed .

Selayar Islands District particularly in rural Lowa village, is an area of relatively high rates of malaria, but until now, there has been no report on the environmental characteristics of breeding habitats and species as well as the density of *Anopheles spp* larvae there. The study aimed to know the spatial distribution and their correlation to characteristics of breeding habitats for *Anopheles spp* larvae density in Selayar Islands village .

METHOD

Location and design of the study

The research was conducted in rural village Lowa, Selayar Islands, with the consideration that the village has a relatively high rate of malaria from the mainland (in 2011, of 67 people examined, 20 had malaria). As a low-lying area, Lowa village has mosquito breeding habitats of *Anopheles spp* breed throughout the year. This due to the shallow groundwater have so if there is a little rain will cause stagnant water, in addition to having a permanent breeding habitats such as lagoon, rivers, swamps, and the wells were flooded throughout the year. This was a cross-sectional study.

Population and sample

Population in this study was all *Anopheles spp* breeding habitat in the village Lowa Selayar Islands District. Sample was the breeding habitats of *Anopheles spp* larvae which can be reached. An accidental sampling was used to determine breeding habitats for this study. Determination of sample points using GPS 12 XL with criteria as follow: 1). All types of breeding habitats of *Anopheles spp* larvae that were found during study. 2). If there were several types of breeding habitat in a sampling site, then any type of breeding habitat was identified. 3). If in a sampling site there are two or more types of the same breeding habitat, then any breeding habitat identified should represent the characteristics of the breeding habitat.

Data Collection

Primary data were obtained through larval survey larva of *Anopheles spp* using dipper on the crackdown with puddles of various habitats by 4 (four) times at each point of larval breeding habitats of Anopheles spp. All larvae that was collected and maintained in the tube until mosquitoes become adult. The adult mosquitoes then taken to the laboratory for species identification. Coordinates of breeding habitat and houses nearby residents as well as the malaria positive patients were taken by Garmin GPS 12 XL. Habitat characteristics were observed in the study include: the type of habitat, the nature of habitat, width of habitat, habitat water depth, water clarity habitat, habitat water color, sea level and sun light of habitat.

Data analyses

Spatial data analysis was done with ArcView GIS. Data point coordinates of breeding habitat was transferred to the sources folder. Subsequent analysis with ArcView to map and display the spatial information/region based on data collected. The characteristic data environment breeding habitat, species and density of *Anopheles spp* larvae through observation sheet further examination/validation data was then inputted and analyzed using SPSS Statistics 17.0. To determine the effect of the dependent variable with the independent variables, we used Kruskall Wallis statistical test, Mann Whitney and Spearman Correlation. To determine the independent variables which most influence on the dependent variable used to multivariate multiple regression analyses.

RESULT

Figure 1 shows four species of larval *Anopheles spp* consisted of *An. vagus, An. subpictus, An. indefinitus, and An. barbirostris*. There are 7 sites ie LOW habitat-1 (swamp 1), LOW-2 (Well), LOW-3 (Ditch 1), LOW-4 (Swamp 2), LOW-5 (River), LOW-6 (Ditch 2), LOW-7 (Pond), where 2 sites has negative *Anopheles spp* larvae in the River and Pond. Distance habitat with houses (the nearest positive and malaria) in the village of Lowa were at a radius of 500-2000 m.

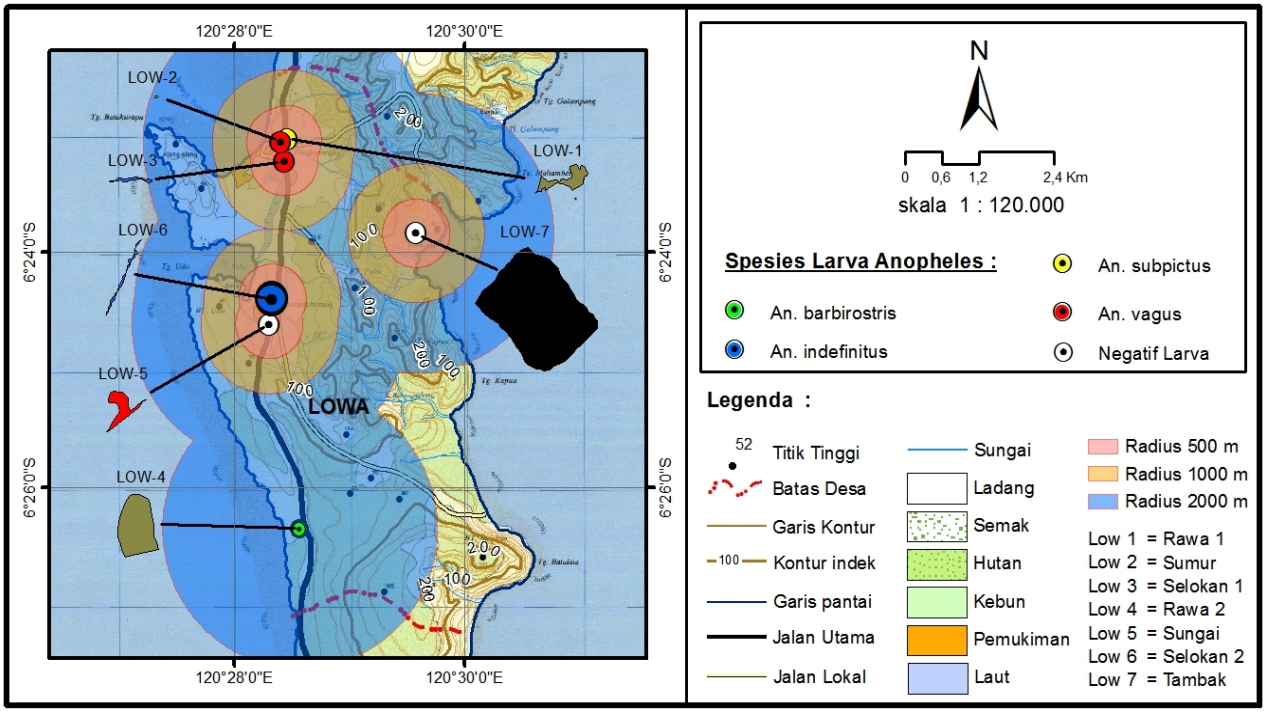


Figure 1. Distribution of breeding habitat, radius between habitat with closer house, positive identified malaria and spesies of larval *anopheles spp* in Selayar Island village (Lowa), 2012

Table 1 shows there were five species found: *An. subpictus, An. barbirostris*, *An. vagus*, *An. indefinitus*. *An. vagus* had two diferent breeding places characteristics.

Tabel 1. Characteristics of breeding habitat dan spesies larva *anopheles spp* in Selayar Island District

No	Variabel	Tipe Habitat						
		Swamp 1	Swamp 2	Well	Ditch 1	Ditch 2	River	Fish pond
1.	Habitat nature	Permanen	Permanen	Permanen	Permanen	Permanen	Permanen	Permanen
2.	Habitat wide (m2)	781,62	615,76	44,05	514,52	914,33	1602,22	10.632,62
3.	Average Water Depth (cm)	27	26	275	13	15	11	145
4.	Water Clarity	Turbid	Turbid	Turbid	Clear	Turbid	Clear	Clear
5.	Water color	Brownish	Red Brownish	Black	clear	Brownish	clear	clear
6.	Habitat Sea level (m)	5	12	10	9	11	4	9
7.	Water flow	Stagnan	Stagnan	Stagnan	Statis	Statis	Dinamis	Stagnan
8.	Habitat Sun light	Half sunny	Shady	Sunny	Half Sunny	sunny	Half Sunny	Sunny
9.	Larvae Spesies	*An. subpictus*	*An. barbirostris*	*An. vagus*	*An. vagus*	*An. vagus, An. indefinitus*	No larvae	No larva

Table 2 showed density of larvae *Anopheles spp.* was influenced by habitat type (p=0.009), depth of water (p=0.000), water clarity (p=0.002), water color effect (p=0.004), habitat altitude (p=0.03), and habitat width (p=0.004). As to habitat width, its r value (r=-0.53) indicated that wider breeding habitat has higher density of *Anopheles spp* larvae. Sun lighting on habitat also influenced larval density (p=0.03). While nature of habitat (p=0.254) and water flow (p=0.07) had no effect on larval density of *Anopheles spp*.

Tabel 2. Breeding habitat characterizaticsand larval density of *Anopheles spp* in Selayar Island District

No.	Variabel	Mean Larva Density	Deviation standard	r value	p value	Statistical Test
1.	Habitat type:					
	- Swamp 1	0,05	0,05			
	- Swamp 2	0,07	0,04			
	- Well	0,02	0,02	*	0,009	*Kruskall Wallis*
	- Ditch 1	0,09	0,14			
	- Ditch 2	0,02	0,02			
	- River	0,00	0,00			
	- Fish pond	0,00	0,00			
2.	Habitat Nature:					
	- Permanen	0,04	0,06	*	0,254	*Mann Whitney*
	- Temporer	0	0			
3.	Habitat Wide	*	*	-0,53	0,004	*Spearman Correlation*
4.	Water Depth	*	*	0,684	0,000	*Spearman Correlation*
5.	Water Clarity:					
	- Clear	0,02	0,01	*	0,002	*Mann Whitney*
	- Turbid	0,05	0,07			
6.	Water Color:					
	- Clear	0,002	0,007			
	- Brownish	0,05	0,08	*	0,004	*Kruskall Wallis*
	- Blackish	0,02	0,02			
	- Red Brownish	0,07	0,04			
7.	Sea level Habitat	*	*	0,42	0,03	*Spearman Correlation*
8.	Water Flow:					
	- Dinamis	0,00	0,00	*	0,07	*Kruskall Wallis*
	- Statis	0,06	0,10			
	- Stagnan	0,03	0,04			
9.	Sun light Habitat:					
	- Sunny	0,01	0,02	*	0,03	*Kruskall Wallis*
	- Half	0,05	0,09			
	- Shady	0,07	0,04			

* = not count

Table 3 revealed there was no characteristics that dominantly influenced *Anopheles spp.* larval density (p >0.05).

Tabel 3. Multiple regression analyses

No.	Variabel	*t*-test	*p-value*	Method
1.	Habitat Type	-1,624	0,123	*Enter*
2.	Habitat Width	1,419	0,174	*Enter*
3.	Water Depth	0,673	0,510	*Enter*
4.	Water Clarity	1,024	0,320	*Enter*
5.	Water Color	0,089	0,930	*Enter*
6.	Habitat Sea level	1,967	0,066	*Enter*
7.	Habitat Sun Light	0,290	0,776	*Enter*

DISCUSSION

Mapping the spatial location of larval breeding habitats of Anopheles spp in rural Lowa counties Selayar Islands was based on the coordinates of GPS (Global Positioning System) from each sampling point. Larval habitats of Anopheles spp were mostly found in Swamp and Sewers. This due in Lowa village nearly all marsh inundated by water as well as sewer contained in each of the houses. This is consistent with the primary data obtained from the Forest Service district Selayar Islands which shows that the location of this study is three- quarters of the forest area.

Distance from habitats to houses (the nearest positive and malaria) in the village of Lowa was 500-2000 m. This suggests that most of the settlements in rural Lowa is an area that is very prone to malaria infection, therefore it is not surprising that ly in Lowa village in 2011 there found 67 patients with positive malaria by 20 people [10]. This is consistent with Hardiman [6] in Mamuju district that showed by 1 kilometer buffer area of malaria, there are mosquito breeding habitats. Most settlements are in areas highly vulnerable to malaria transmission except residential areas to the west and south of the village Karampuang and residential areas to the south village Rimukku.

Bivariate analysis found that there was the influence of habitat type on the density of larval *Anopheles spp*. Density of Anopheles spp larvae that most are in the gutter habitat. This suggests that each species of *Anopheles spp* have favored place to do breeding. In accordance with this research discovery *An. barbirostris* larvae on marsh habitat, a habitat which is generally favored by the Anopheles species. Another study of Jastal [11] found the species *An. subpictus* the neglected fish ponds, a pool of brackish water in the sun directly. On the other hand, there was no influence of the nature of the density of larval habitats of Anopheles spp. This is because the survey data in rural Lowa, all habitats found is a permanent habitat totaling 7 sites. So it is very difficult to see the influence of the nature of the density of larval habitats of Anopheles spp.

Width of habitat influence larval density of Anopheles spp. This study found that the wider breeding habitat, where the density of larvae of Anopheles spp less. This suggests that the wide of habitat is not a barometer of larval density is also higher. Water depth influence on the density of larval Anopheles spp. Statistical test results indicated that the deeper the water depth breeding habitat, the larvae of Anopheles spp density decreases. In general, the waters of life-sustaining water creatures are only the surface layer. Increasingly in the lower diversity of life and the lower the carrying capacity of life [12]. Water clarity influenced the density of larval *Anopheles spp*. This can happen because of certain *Anopheles* species prefers a particular water to lay eggs, but there are also female mosquitoes usually lay their eggs anywhere as long as there is water: fresh water/salt crystal clear/clean or turbid/foul and trace footprints [13].

Water color affect on the density of larval Anopheles spp. The highest density of larvae in the water habitats are maroon. Brownish red color is influenced by many factors other rainwater that seeps from the mainland into the habitat, but it is also due to the flora (trees that fall into the category simbuta Rhyzophora/mangrove tree) in which the roots spread and secrete sap reddish color [14].

Habitat altitude influence on larval density of Anopheles spp. Statistical test results obtained r values (r=0.42) indicated that the higher places breeding habitat, the larvae of Anopheles spp density decreases. In the fact that any increase in the surface 100 m, followed by 0.5^0C increase in temperature, so if the difference in ground level

the higher the temperature difference is also quite high, so that the influence of other factors [13]. This study is similar to Hardiman [6] that the malaria mosquito breeding habitats in Mamuju District has a tendency or trend in low-lying areas and urban areas.

There was no effect of water flow on larval density of *Anopheles spp*. This could have happened because at the time of the survey, the flow of water in some habitats fluctuate because rainfall is erratic, causing difficulty to observe the density of larvae of each habitat based on water flow. There was the influence of the habitat exposure on larval densities of Anopheles spp. This happens because certain species of Anopheles mosquitoes prefer certain places for breeding. For example, if the place is quite open to the possibility of developing mosquitos were *An. sundaicus*, when the place is pretty shady possibilities can be found *An. barbirostris* [13].

Results of multivariate analysis of the independent variables that affect the density of Anopheles spp larvae, not obtained the most influential factor (dominant) against the density of larval Anopheles spp. This shows that all the factors that affect the density of larvae of Anopheles spp in Lowa village has influence each other is strong enough synergism).

CONCLUSION

We conclude that there are four species of larval *Anopheles spp.* namely *An. vagus, An. subpictus, An. indefinitus, and An. barbirostris*. The type of positive larval habitats of Anopheles spp varied from swamps, wells, and sewers. There were 7 factors that affect the density of Anopheles spp larvae in habitats that are found among other things: the type of habitat with the highest density being in the ditch habitat, habitat, water depth, water clarity, water color, height and lighting habitat habitat. To the eradication of malaria in rural Lowa, not quite with the distribution of insecticide-treated nets, spraying houses/IRS and discovery/treatment of patients. Malaria eradication is effective and efficient in eradicating the Anopheles mosquito larvae and species that act as vectors of malaria in order to break the chain of transmission of malaria in rural Lowa.

REFERENCES

1. Sembel DT. Medical Entomology. Yogyakarta: Andi Publisher; 2009.
2. Harijanto A. Malaria, Epidemiology, Pathogenesis, Clinical Manifestations and Treatment. Jakarta; EGC; 2000.
3. District Health Office of Selayar Islands. Health profile of Selayar Islands. 2010
4. Cailly P. et al. Role of the Repartition of Wetland Breeding Sites on the Spatial Distribution of Anopheles and Culex, Human Disease Vectors in Southern France. Parasites & Vectors 2011;4:65.
5. Gouagna et al. Spatial and Temporal Distribution Patterns of *Anopheles Arabiensis* Breeding Sites in La Reunion Island - Multi Year Trend Analysis of Historical Records From 1996-2009. Parasites & Vectors 2011, 4:121 .
6. Hardiman. Spatial patterns of malaria mosquito breeding habitats in the Mamuju District with Geografis Information Systems (GIS). Hasanuddin University Graduate Thesis: Makassar, 2004.

7. Arsin , A. A. (2004) . Epidemiological analysis of malaria incidence in Pangkajene Islands District South Sulawesi Province . Hasanuddin University Graduate Dissertation : Makassar .
8. Directorate General of Disease Control & Environmental Health . (2009) . Decree of the Minister of Health R.I. No. . 293/MENKES/SK/IV/2009 . Jakarta: Indonesia Ministry of Health.
9. Adrial . (2008) . Fauna of Malaria Vector Mosquito, *Anopheles* in the Areas Around Campus Lemons Manih Andalas University , District of Padang , West Sumatra province . Department of Parasitology Faculty of Medicine, University of Andalas : Padang
10. DHO. (2010). Islands Selayar 2010
11. Jastal . (2003) . Anopheles breeding places . UPF - PVRP Donggala . Ecology Research Center for Health Litbangkes .
12. Mogi , et al . (1995) . Mosquito (Diptera : Culicidae) and Predator Abundance in Irrigated and Rain - Fed Rice Fields in North Sulawesi , Indonesia . Journal of Medical Entomology . Vol . 32 3 : 361-367 .
13. Susanna , D. (2011) . Health Entomology (Health and Endocrine arthropod parasites it contains) . UI Press : Jakarta .

MALARIA AT BANJARNEGARA DISTRICT IN THE LAST FIFTEEN YEARS

Bina Ikawati, Sunaryo
Balai Litbang P2B2 Banjarnegara, Balitbangkes, Kemkes RI
Correspondence: bina@litbang.depkes.go.id

ABSTRACT

Background: Malaria is one of health problem in the world also in Indonesia. The number of malaria cases in Banjarnegara District placed in second position after Purworejo district in Central Java. This study aimed to describe malaria cases in Banjarnegara last of fifteen years by data and literature review.
Method: This was a descriptive study to review malaria cases. Data was obtained from District Health Office of Banjarnegara
Result: Result showed that in last fifteen years malaria in Banjarnegara has been decreasing, but malaria outbreak happened sporadically in some village mainly in middle and south region. *Plasmodium vivax* found quite a lot recent years. Strengthening cross program and sector, community empowerment and government commitment need to support malaria elimination in Central Java by 2015 that would support malaria elimination in Indonesia by 2030.
Keywords: Banjarnegara, malaria, elimination

BACKGROUND

Malaria is one of mosquitoes transmitted diseases and became priority. This disease caused by Plasmodium. Four type of plasmodium found in Indonesia are *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae* [1]. Newest invention reported new Plasmodium, *P. knowlesi*, which was found in Borneo (Kalimantan) [2]. As much 98% Plasmodium found in Indonesia were *Plasmodium falciparum* and *P. vivax* [1]. Malaria is transmitted by *Anopheles spp*, but not all species of Anopheles could become malaria vector. Besides, a vector in one area might not serve as vector in other areas. Species of Anopheles could be a vector if have longevity so Plasmodium cycle in mosquito from gametosit to sporozoit done, vector density high enough, contact with human, succeptible to paracite and there were source of diseases. Research showed Anopheles that confirmed as vector in Banjarnegara District, Central Java were *An. aconitus, An. maculatus* dan *An. balabacensis* [3].

Malaria control and eradication need national commitment, regional and also global as contained in *Millenium Development Goals* / *MDGs*. World Health Assembly 60 meeting in 2007 yielding global commitment about Elimination Global of Malaria to every country. In Indonesia, malaria elimination program based on decision Ministry of Health Republic of Indonesia No. 293/MENKES/SK/IV/2009, April 28th 2009, about malaria elimination in Indonesia and Letter from Domestic Ministry Republic of Indonesia to all governor and regent in Indonesia No. 443.41/465/SJ, February 8th 2010, about guidance of malaria elimination in Indonesia. In central Java Province targeted that malaria will be eliminate by 2015 [4]. At 2012 in Central there were 8 Sub district with malaria indegenous and import cases, 20 Sub district with import cases and 7 Sub

district with no malaria cases. Malaria cases in Banjarnegara District placed rank 2 in Central Java after Purworejo with API 0,14‰ in 2012 [5]. This article describeb malaria cases in Banjarnegara last of fiveteen years and recording research that ever done in Banjarnegara.

METHOD

Literature and data review were used as mode to write this article.

RESULT

Banjarnegara District is located in $7^0$12'-$7^0$31' latitude and $109^0$20'-$109^0$45' longitude. This district had 106,970,997 Ha land, consisted of 13.9 % agriculture ricefield area, 52.2 % non agriculture ricefield area, and 33.9 % others. This district is a hilly area in middle of Central Java, north area of this district had surging and steep relief, in the middle level off area, and in south had steep relllief [5].

Annual Parasite Incidence (API) malaria in Banjarnegara since 1995 until 2012 showed increase from 1995 until 2002 and tend to down until 2012, little different with API in central Java at 2000-2003. At the year of 2000 API in Central Java reaching top while in Banjarnegara decrease. At 2002 API in Banjarnegara reaching top while in Central Java decrease (Figure 1).

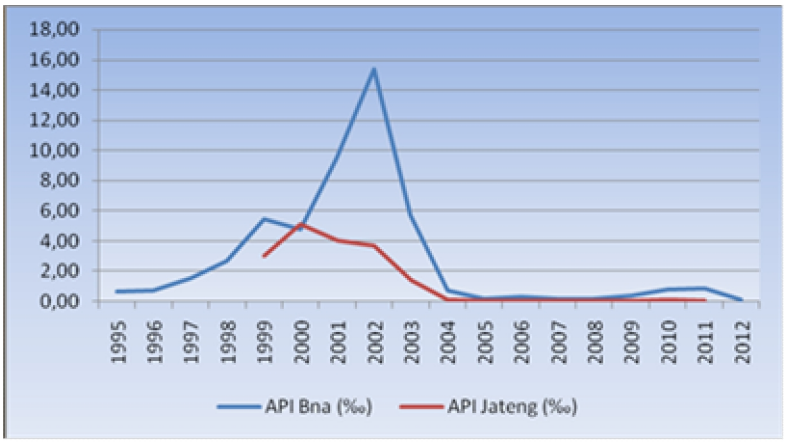


Figure 1. Annual paracyte incidence (API) malaria in Banjarnegara 1995-2012
Data source: Banjarnegara District Health Officer 2006, 2013

In the last six years, south areas of Banjarnegara had high and middle malaria incidence according to API. Subdistricts with API >5‰ mostly found in 2001-2003, while in 2004-2009 none of subdistricts had API >5‰. However, in 2010 there were two subdistricts (Punggelan and Pagedongan) with API >5‰ (High Case Incidence/HCI) and one subdistrict (Banjarmangu) with API 1-5‰ (Middle Cases Incidence/MCI). In 2011 malaria status at Banjarmangu turned to HCI. There was new MCI area (Wanadadi subdistrict), while Pagedongan remained constant (HCI). In 2012 malaria status in Punggelan and Wanadadi became MCI, while Banjarmangu remained HCI (Figure 2).

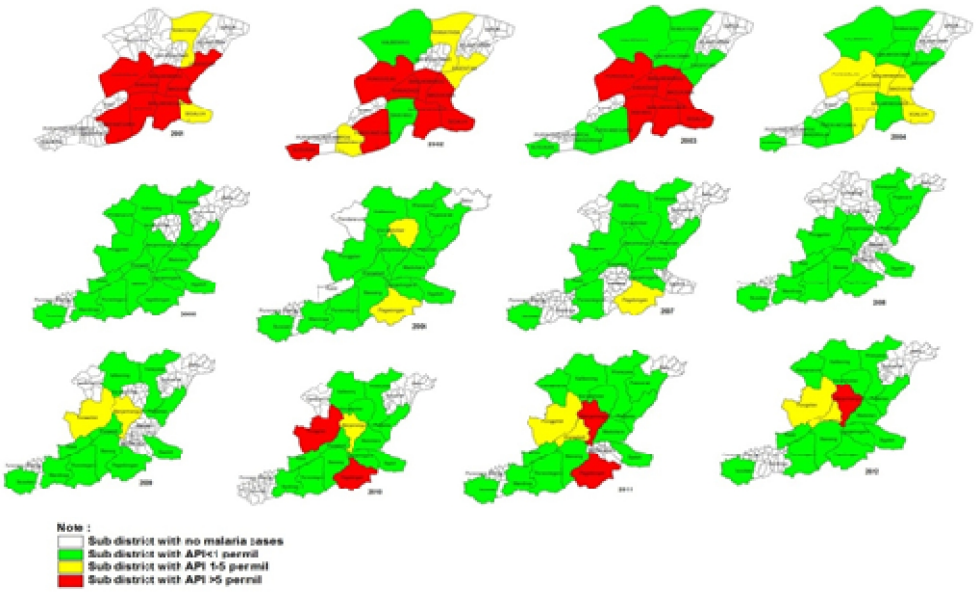
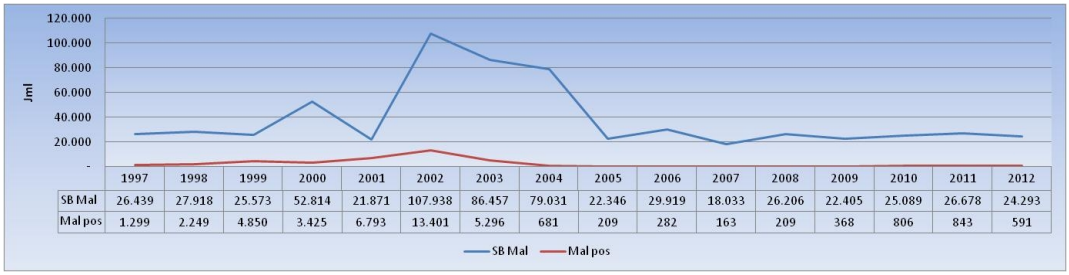


Figure 2. Malaria endemicity strata based on API by subdistrict in last of 12 years
Data source in making map: Banjarnegara District Health Officer 2006, 2013

Distribution of malaria focused in middle of Banjarnegara district (Punggelan, Wanadadi, Banjarmangu and Madukara Sub district) and south of Banjarnegara district (Pagedongan, Bawang, Purwonegoro Sub district). Figure 3 showed comparation of blood slide examinated and slide positive malaria (malaria cases) was in parallel. The different only happen in 2001 when blood slide examinated decreases but malaria cases increased.



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
SB Mal	26.439	27.918	25.573	52.814	21.871	107.938	86.457	79.031	22.346	29.919	18.033	26.206	22.405	25.089	26.678	24.293
Mal pos	1.299	2.249	4.850	3.425	6.793	13.401	5.296	681	209	282	163	209	368	806	843	591

Figure 3. Blood slide examinated and slide positive of malaria
in Banjarnegara 1997-2012
Data source: Banjarnegara District Health Officer 2006, 2013

"Gebrak malaria" that had been done by Banjarnegara goverment in 2002 that hold cross program and sectoral seen to depress malaria transmission. Juru Malaria Desa had contributed to found malaria by Active Case Detection specially in endemic malaria area. In 2002 there were 181 Juru Malaria Desa with contract system and in 2010 only 118 Juru Malaria Desa most of that had been an civil servant. Amount of decreasing because a little number was retired and the other placed in new job outside of health area after became a civil servant [6,7].

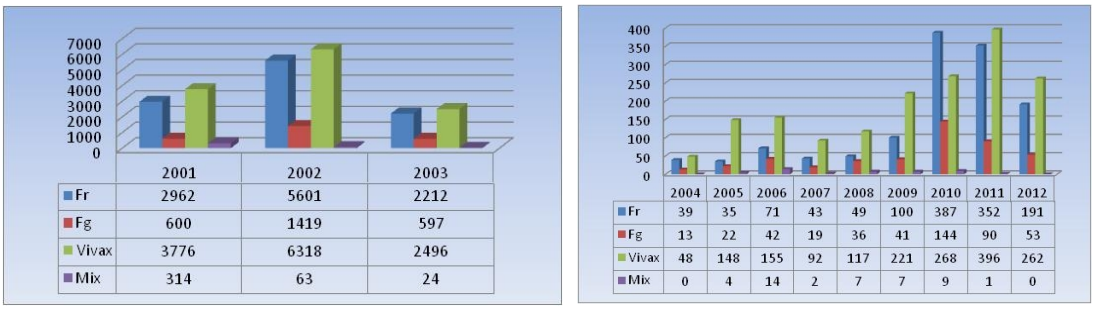
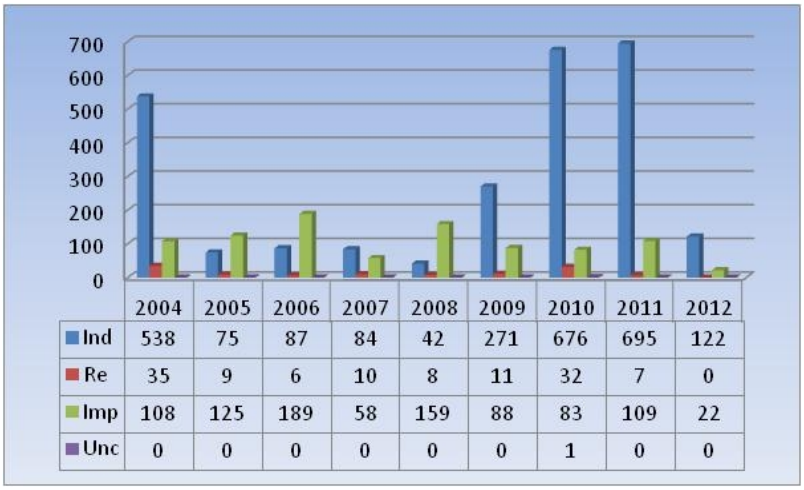


Figure 4. Proportion of Plasmodium in Banjarnegara 2001-2012
Data source : Banjarnegara District Health Officer 2006, 2013

Figure 4 showed that *P. vivax* was dominated and *P. falciparum* (ring and gamet) dominated in certain years. Indegenous malaria cases were dominated in years of 2004, 2009 until 2012, while import cases dominated in 2005-2008. But that way in many cases indegenous malaria cases triggered by import cases (figure 5).



Note : Ind=indegenous, Re=relapse, Imp=impor, Unc=unclasification

Figure 5. Infection status of malaria cases in Banjarnegara district at 2004-2012
Data source : Banjarnegara District Health Officer 2006, 2013

Distribution of malaria vector covered 70% area of Banjarnegara. The complete distribution seen in figure 6.Vector variation (3 species vector) found in 601-900 m asl. Only two species that were found at height <or >601-900 asl *An. aconitus* and *An. maculatus* (figure 7).

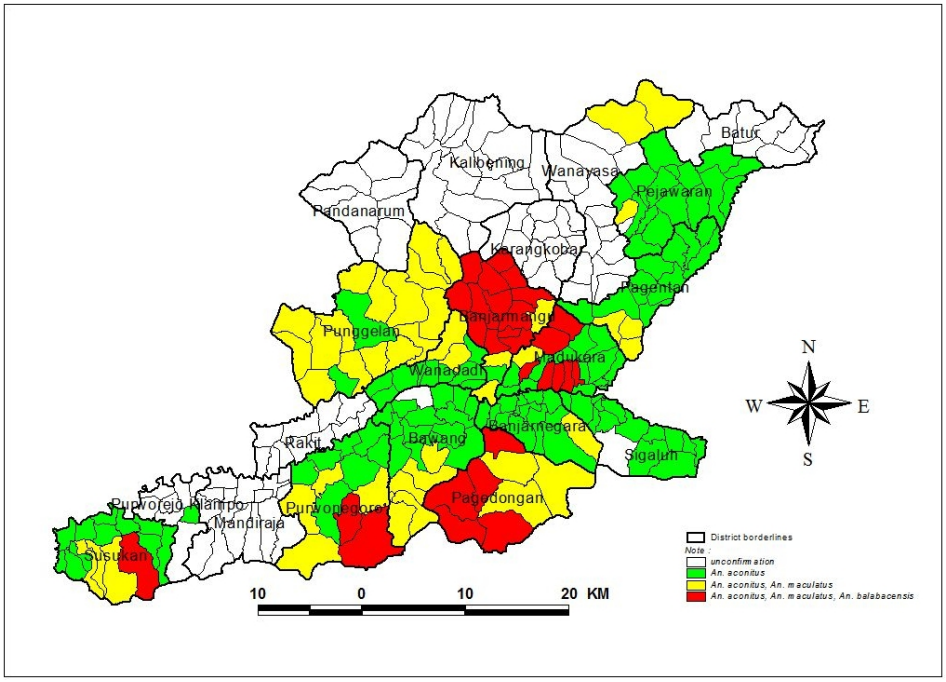


Figure 6. Map of malaria vector distribution in Banjarnegara

Data source: Luo D in Banjarnegara Field Vector Control Station 2001 unpublished data, Banjarnegara Animal Borne Diseases Control Research and Development Unit 2013

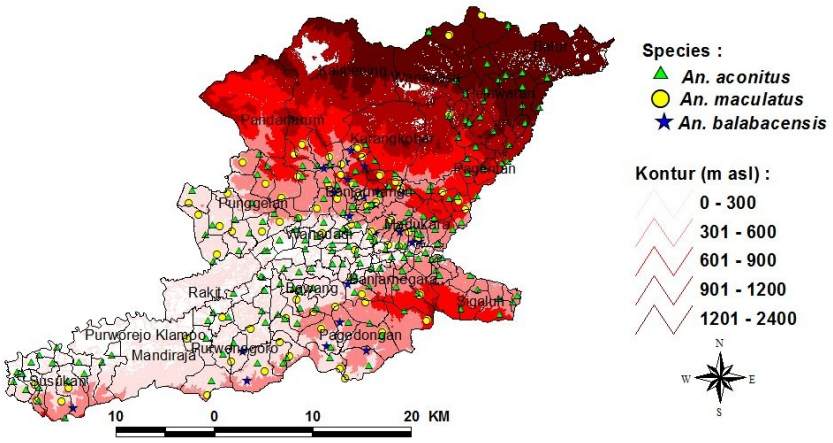


Figure 7. Malaria vector in Banjarnegara based on height

Data source : Banjarnegara Field Vector Control Station 2001, Banjarnegara Animal Borne Diseases Control Research and Development Unit 2013

Based on land use malaria vector mostly found in salak garden area. However, study in 2001 failed to prove relationship between salak garden with vector bionomic. Malaria vector in salak garden area influenced by existence of mosquito breeding places that depend on treatment of salak garden. Drainage in salak garden with wellspring had big influenced to made of breeding compare with irigation of cisstern of rain. This

research suggest community to clean up salak garden environment and decrease frond of salak to keep humidity and illumination [8]. Yunianto B, Bina I and Sunaryo research expressing that *Anopheles balabacensis* found in salak garden dominantly. Breeding place of *An. balabacensis* found in wellspring near salak garden [9]. Species variation of Anopheles in salak garden more variety than in non salak [10]. Malaria vector found in southwest part of Banjarnegara in ricefield irrigation. Nalim [11] revealed that mosquitoes habitats decreases, especially *An. aconitus*, after adding of consumption fish together with *Poecilia reticulata* in ricefield. This eventually would decrease malaria cases. In this case, malaria in Pagak Village drasticly decrease and no malaria outbreak was reported. In fact, malaria vector remained succesfully controlled in that area [11].

Land use of Banjarnegara and existence of 70% area with malaria vector supported transmission of malaria, if there was one sufferer malaria with gamet phase the probability of transmission was higher specially in area with 3 species vector. To prevent malaria transmission from import cases goverment socialized migration surveilance which its content people from or going to go to out of Java Island should be report to officier of health that will be checked for malaria. As implementation in village level there were 3 village as sample of village regulation about migration surveilance, that had been done in Sipedang Village Banjarmangu Sub district, Petuguran Village Punggelan Sub district and Kebutuhduwur Village Pagedongan Subdistrict in 2012 [12].

ICDC malaria vector observed in 2001-2003 that done in Pagelak Sub village, Kraminan Village, Madukara Sub district showed *An. aconitus* found all night, increased in February reach the top in April and decrease in August, increases in October decreases in January. Relative density outdoor higher than indoor. *Anopheles balabacensis* most found after midnight in fluctuative density. Relative density indoor and outdoor much the same to. Relative density of *An. aconitus* and parousity of *An. aconitus* is not followed by increases of malaria cases [13].

Study on community participation in malaria control using *Bacillus thuringensis* H-14 local clan was performed in Gunungjati village. The result showed knowledge about malaria and malaria control using *Bacillus thuringensis* H-14 increased after training. As much 80% society agree to use BTI H-14 in breeding places habitat, 60% ready to prepare their own coconut as growing media of BTI and 64% agree to keep BTI H-14 and equipments had been given [14]. However, longterm evaluation had not be done to monitor BTI H-14 maintenance.

Succeptibility test of malaria vector ever been done in 2003 to *An. aconitus* in Pagelak Village Madukara Sub district. Impregnated paper used was containing permethrin 0,75% and deltamethrin 0,05%. This research showed that vector resistence of insecticide tested in verification level [15]. Banjarnegara also one of location in resistensi og chloroquin with result in 1989 some of *P. falciparum* had chloroquin resistent 71,4%(10/14) sensitive case or R1 late, 21,4%(3/14) case with R2 and 7,1% (1/14) R3 case [16].

DISCUSSION

Malaria cases in Banjarnegara District last of fiveteen years was decreased. This trend had similarity with malaria trend in Central Java Province, probably that because of Banjarnegara District as high contributor of malaria cases in Central Java. Malaria

problem concentred in middle and sout area of Banjarnegara. In the year of 2001 until 2003 there were much Sub district with HCI category specially in middle and south area of Banjarnegara. Annual Blood Examination rate in 2001-2003 showed higher than after that years. Probably one of caused increases malaria at that years was the high of blood examination rate, as implementation program of Intensive Community of Diseases Control (ICDC) with loan of Asian Development Bank that done at that year. Malaria distribution linier with existence of malaria vector in Banjarnegara, in middle and south area of Banjarnegara three kinds of species vector found.

Plasmodium vivax that found dominantly in Banjarnegara caused threat of malaria transmission if uncomplete medication. This becaused *P. vivax* had hipnozoit phase, and long incubation.[1] The found of Plasmodium falciparum in gamet phase compare with Plasmodium falciparum ring about 1:3 every years showed different two sides. Gamet phase is the phase of plasmodium that ready to transmitted, gamet phase had been found showed that source of transmission founded. In the other side it showed late of case detection. Impor case of malaria dominated in 2005-2008, before and after that year indegenous cases most found. One thing that must be noted that indigeneous malaria cases mostly caused by impor as index cases. The high number of malaria impor cases found would be caused a little indigeneus malaria cases found.

Banjarnegara district had 70% reseptive area for malaria transmission. The existence of three malaria vector in middle and south area of Banjarnegara would be caused malaria transmission more higher and spread in that area and proved in malaria cases trend 2001-2012. That area need more attention in malaria cases surveilance. The existence of Juru Malaria Desa as observed malaria cases specially in village with malaria problem(village with HCI or MCI criteria) still needed to observed malaria. To supporting malaria elimination activity must be stressing to found impor case. Regulation about surveilance migration of malaria must be extended and well accepted by community specially in middle and South area of Banjarnegara. This program need to socialized in community so not just a regulation but implemented in community. To conducting all of that Health District Officer cannot doing by itself, cooperation and commitmen of goverment needed, and also cross program and sectoral help. Thereby expected that trend of malaria cases more to impor cases and will be reduce or there no indigenous cases (include indegenous with impor as index cases), in this phase malaria elimination reached.

Study in South Africa describe pivotal aspect in malaria elimination physical (temperature and humidity), social (migration patterns), economic (quality and housing stock) and political (regional collaboration). Strengthening to control malaria transmission factor caused this area has decline remarkably over the past of 12 years.[17] The high number of *P. vivax* should be aware because from research showed that systemic parasitic and bacterial infections, but not viral infections, can activate *P vivax* hypnozoites. Specific components of the host's acute febrile inflammatory response, and not fever alone, are probably important factors in the provocation of a relapse of vivax malaria [18].

CONCLUSION

In last of fifteen years malaria cases in Banjarnegara trend to decreases or back to condition before 1999. Plasmodium vivax was dominated. Middle and south area of

Banjarnegara must be allert from malaria. Found of import cases would be impress malaria transmission. To support malaria elimination encourage to found of malaria impor must be improved from health programmer, health officer and community conciousness Juru Malaria Desa still needed specially village in middle and south area of Banjarnegara.

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REFERENCES

1. Departement of Health Republic of Indonesia. Directorat General of Communicable Diseases Control and Environmental Helath. Animal Borne Disease Control Directorat. Malaria Parasitology Module 2. Jakarta. 2001.Indonesian.
2. Figtree M, Rogan L,Lisa B, Tom K, Sonia M, Merrill U, et al. Plasmodium knowlesi in human, Indonesian Borneo. Emerging Infectious Diseases.www.cdc.gov/eid. Vo. 16 No. 4 April 2010 Pages 672-4.
3. Widyastuti U. Verification of Malaria Vector in Area of ICDC ADB Project in Central Java using ELISA methode. Final Report of ICDC Research. Salatiga Vector and Reservoir of Diseases Research Unit. 2003.Indonesian.
4. Central Java Province Health Office. Policy of malaria elimination in Central Java Province. Presented in malaria elimination meeting in endemic malaria district. Banjarnegara, September 10th2013.Indonesian.
5. Statistic of Banjarnegara Regency. Banjarnegara in Figures 2012. Banjarnegara. 2013. Indonesian
6. Banjarnegara District Health Office. Malaria situation in Banjarnegara at 2001-2006. Presented in Scientific Meeting Banjarnegara Animal Borne Diseases Control Research and Development Unit.Banjarnegara, May 30-31th 2006. Indonesian.
7. Banjarnegara District Health Office. Malaria situation in Banjarnegara District. Presented in Elimination of Malaria meeting. Banjarnegara September 10th 2013.Indonesian.
8. Kristi N. Relationship between malaria and vector bionomic in Salak garden at Kendaga and Sijeruk Villages Banjarmangu Sub district Banjarnegara District. Banjarmangu II Public Health Center, Banjarnegara. 2000. Unpublished research report. Indonesian.
9. Yunianto B, Bina I, Sunaryo. Study of Ecology *Anopheles balabacensis* in Salak and or without salak area in Banjarnegara District. Balaba Vol 5 No. 2 December 2009. Pages 1-6. Indonesian.
10. Widiastuti D, Bambang Y, Bina I. Variability of Anopheles mosquitoes species in salak and non salak area in Banjarnegara District. Edisi 002 Nomor 01 Year II June 2006. Pages 12-4.Indonesian

11. Nalim S, Damar TB, Arief H dan Elias W. Control Demonstration of the Ricefield Breeding Mosquito Anophele aconitus Donitz in Central Java using Poecilia reticulata through community participation. 3 Field Trial and Evaluation. Bul. Penelit.Kesehat 16(1). 1988. Pages : 6-11
12. Banjarnegara District Health Office. Malaria Migration Surveilance in Banjarnegara District. Presented in National Seminar of Malaria. Banjarnegara Polytechnic. Banjarnegara 6 October 2012. Indonesian
13. Central Java Field Vector Control Station. Longitudinal Survey of Malaria Vector Report in 4 ICDC District, Central Java Province. Health Departement Central Java Regional Office. Unpublished report. 1999/2000. Indonesian.
14. Trapsilowati W, Blondine Ch. Community participation in Malaria vector control using Bacillus thuringensis H-14 local clanin Banjarnegara District, Central Java. Media Litbang Kesehatan Vol. XX Nomor I Year 2010. Pages : 26-32.Indonesian.
15. Widiarti, Damar TB, Barodji and Mujiono. Succeptibility test of *Anopheles aconitus* and *Anopheles maculatus* to Syntetic Pyrethroid insecticide in Central Java and Yogyakarta Province. Jurnal Ekologi Kesehatan Vol 4 No. 2 Agustus 2005. Pages : 227-32Indonesian.
16. Sekartuti, Arbani DR, Romzan A, Tjitra E, dan Rheny M. Masalah Plasmodium falciparum resisten to kloroquin and or another malaria drug in Banjarnegara District, Central Java. Majalah Kedokteran Indonesia 1994. 44(6). Pages 377-83. Indonesian.
17. R Maharaj, J Raman, N Morris, D Moonasar, DN Durrheim, I Seocharan,et al. Epidemiology of malaria in South Africa : from control to elimination. Oct 2013.Vol 103 No. 10 (Suppl 2) SAMJ. Pages: 779-83
18. Shanks D, Nicholas JW. The activation of vivax malaria hypnozoites by infectiousdiseases.http://www.sciencedirect.com/science/article/pii/S1473099137000951. Accessed Sept 8th 2013.

DISTRIBUTION OF *ANOPHELES* AS SUSPECT OF MALARIA VECTOR IN LAHAT DISTRICT, PROVINCE OF SUMATERA SELATAN

Budiyanto A, Taviv Y, Ambarita LP
Loka Litbang P2B2 Baturaja
Correspondence: anifbdt@litbang.depkes.go.id

ABSTRACT

Background: Lahat district is one of the malaria endemic areas in South Sumatera Province. Number of cases of malaria each year in Lahat district fluctuated between 19.8% o to 29.7%. Information of distribution of malaria vector in endemic areas is needed to determine the appropriate methods of malaria control program. It is not yet known how the distribution of malaria vector in Lahat district. The aim of this study was to determine the distribution of malaria vector in Lahat district.
Methods: The study used a survey design. Adult mosquito's collection carried out in 11 location using human landing collection and resting collection methods. Mosquito's collection carried out by 6 collectors start from 18.00 pm until 06.00 am in 6 selected houses.
Results: A total of 367 *Anopheline* mosquitoes within 16 species were captured: *Anopheles sinensis, An. nigerrimus, An. barbirostris, An. maculatus, An. schufneri, An. barbumbrosus, An. vagus, An. kochi, An. annularis, An. An. separatus, An. phillipinensis, An. tesselatus, An. umbrosus, An. aconitus, An. letifer* and *An. leucosphyrus* group. Among all species collected *An. vagus* and *An. kochi* were the predominant species collected. Of 240 mosquito's sample, there were no sporozoit detected by PCR method.
Conclusion: *An. nigerrimus* and *An. letifer* are vector of malaria in South Sumatera Province were found distributed in some areas of Lahat districts. Further research is needed to determine the bionomic of both vector species.
Keywords: malaria, *Anopheles*, entomology survey

BACKGROUND

Malaria is one of infectious disease is still a public health problems in the world, including Indonesia. This disease causes high mortality of infants, toddlers and pregnant mothers. Every year more than 500 millions of world population infected with malaria and more than 1,000,000 people died. Indonesia is one of the countries that is at risk against malaria. In the year 2007 in indonesia there were 396 district endemis malaria from existing, 495 district with an estimate of around 45 % of the population live in areas at risk of contracting malaria [1]. Malaria is one of the indicators of the targets of the millennium development goals (MDGs), which targeted to stop the spread of events and reduce the incidence of malaria in the year 2015 viewed from an indicator of the decrease in numbers in pain and the number of malarial deaths. In the strategic plan health ministry 2010-2014 year malaria control is one disease is pain to reduce the rate of two to one per 1,000 inhabitants [2].

Lahat District is one district in South Sumatera province that is malaria endemic area. It has highest case of malaria in the province [3]. Based on the case report in

period of June to December 2011, there were 5.463 malaria suspects whose blood was examined. The result showed as many as 898 positive malaria (slide positive rate/SPR = 16.5 %) [3]. The number of malaria case in the county of service every year experienced fluctuations between 19.8 ‰ until 29.7 ‰. While the number of cases of malaria based on examination the laboratory than last year until 2011 is worth 5.49 ‰ and 3.55 ‰ [4].

In epidemiology problems the case of malaria in the county of service involving multiple factors determiner very complex, epidemiological which is parasitic agent diseases (*Plasmodium falciparum, P. vivax*), the host (human and Anopheles mosquito as vector), and environmental factors influence it including socioeconomic and behavior of local residents. Epidemiological information on multifactors decider of vectors bionomics and environmental is still lacking. This information is needed as one of means in developing specific, effective, and efficient way to control malaria that can be applied with satisfactory results in the endemic area of malaria.

In order to determine strategies for the eradication of malaria in Lahat, hence the need for comprehensive efforts and intensive data by utilizing the bionomic of malaria vector in endemic malaria regions are accurate and specific, so it can be determined the type and pattern of intervention that is effective and efficient. To date is not yet known how the distribution of Anopheles spp and Anopheles bionomic in Lahat. The purpose of this research was to describe the distribution of the Anopheles mosquito, and its bionomic the Lahat.

METHOD

Adult mosquito was catched from 11 locations, each for 12 hours from 06.00 pm to 06.00 am. The method of catching used man bait, consisted of 3 persons inside and 3 persons outside houses. We selected 6 houses in each location (3 houses to catch mosquitoes outdoors and other 3 to catch mosquitoes in the house).

RESULT

Result of bionomic survey in several locations found 367 infected Anopheles mosquito, consisted of 16 species: *An. nigerimus, An. vagus, An, barbirostris, An. teselatus, An. barbumbrosus, An. umbrosus. An. sinensis, An. maculatus, An. schufneri, An. kochi, An. anularis, An. letifer, An. leucospirus, An. separatus, An. philipinensis, An. aconitus*. Confirmation of mosquito as vector was done by PCR test at the Eijkman Jakarta. From 240 samples of mosquitoes that is examined, all was negative of sporozoites. Results of the survey conducted at few locations Entomology note that peak biting Anopheles mosquito varies, and the method of catching up with the number of Anopheles mosquitoes can also vary in.

Table 1. *Anopheles* species found in Lahat district

Desa/Kelurahan	Kecamatan	Jenis *Anopheles*	MHD				MBR	K	KN	D
			UOL	UOD	RL	RD				
Sukoharjo	Kikikm Timur	*An. Sinensis*	0,00	0,02	0,00	0,00	0,17	0,08	100	8
Mutar Alam Baru	Kota Agung	*An. Nigerimus*	0,02	0,00	0,00	0,00	0,17	0,08	11,11	0,01
		An. Barbirostris	0,04	0,00	0,00	0,08	0,33	0,25	33,33	8,33
		An. Maculatus	0,02	0,00	0,16	0,00	0,00	0,25	33,33	8,33
		An. Schufneri	0,02	0,00	0,00	0,00	0,00	0,08	11,11	0,93
		An. barbumbrosus	0,00	0,00	0,00	0,08	0,00	0,08	11,11	0,93
Sukajadi	Pseksu	*An. Vagus*	0,00	0,00	0,25	0,25	0	0,25	100	25
Talang Jawa Utara	Lahat	*An. Vagus*	0,00	0,02	0,00	0,00	0,17	0,08	100	8
Purwaraja	Palembaja	*An. Vagus*	0,02	0,00	1,14	0,08	0,17	0,58	60,71	35,42
		An. Kochi	0,02	0,00	0,65	0,08	0,17	0,42	35,71	14,88
		An. Annularis	0,00	0,00	0,08	0,00	0,00	0,08	3,57	0,30
Karang Baru	Lahat	*An. Vagus*	0,00	0,02	0,00	0,00	0,17	0,08	100	8
Tanjung Bulan	Tanjung Sakti Pomo	*An. Nigerimus*	0,02	0,00	0,00	0,00	0,17	0,08	25,00	2,00
		An. Sinensis	0,02	0,00	0,00	0,00	0,17	0,08	25,00	2,00
		An. Separatus	0,00	0,00	0,00	0,08	0,00	0,08	25,00	2,00
		An. Vagus	0,00	0,00	0,00	0,08	0,00	0,08	25,00	2,00
Lebak Budi	Merapi Barat	*An. Vagus*	0,50	0,41	4,00	1,00	7,33	0,92	98,31	90,11
		An. Nigerrimus	0,02	0,00	0,00	0,00	0,17	0,08	0,85	0,07
		An. Barbirostris	0,00	0,02	0,00	0,00	0,17	0,08	0,85	0,07
		An. Philipinensis	(1 ekor/light trap)							
Karang Rejo	Lahat	*An. Vagus*	0,00	0,02	0,49	0,00	0,17	0,33	53,85	17,77
		An. Tesselatus	0,00	0,00	0,16	0,00	0,00	0,17	15,38	2,61
		An. Umbrosus	0,00	0,00	0,08	0,00	0,00	0,08	7,69	0,62
		An. Letifer	0,02	0,00	0,00	0,00	0,17	0,08	7,69	0,62
		An. leucosphyrus group	0,04	0,00	0,00	0,00	0,34	0,17	15,38	2,61
Gedung Agung	Merapi Timur	*An. Nigerrimus*	0,04	0,00	0,00	0,00	0,34	0,08	13,33	1,07
		An. Vagus	0,04	0,00	0,37	0,00	0,34	0,33	40,00	13,2
		An. Philipinensis	0,02	0,00	0,16	0,00	0,17	0,25	20,00	5,00
		An. Sinensis	0,06	0,00	0,08	0,00	0,50	0,25	26,67	6,67
Bunga Mas	Kikim selatan	-								

MHD = Man Hour Density UOL = Umpan Orang Luar UOD = Umpan Orang Dalam
RL = Resting Luar RD = Resting Dalam MBR = Man Biting Rate
K = Kekerapan KN = Kelimpahan Nisbi D = Dominansi

DISCUSSION

Spot survey results obtained 16 Anopheles mosquito species. Two species are confirmed as vectors of malaria in the Province of South Sumater, namely *An.*

nigerimus and *An. letifer*. *An. nigerrimus* caught in six villages, while *An. letifer* only caught in a village. The density of both species appertain was fairly low. We also caught *An. maculatus* and *An. leucosphyrus* each at one village. Both of these species have habitats in the region of the hills. This is in accordance with previous study [6]. Research conducted by Bogh and Comrades in hills of coffee plantation at Regency of Ogan Komering Ulu South Sumatra declared this species as a potential vector because they contain sporozoit on the salivary glands of mosquitoes through test ELISA [7].

Some Anopheles mosquitoes, which are vectors of malaria, were found in several locations outside of South Sumatra Province, as *An. aconitus* in Lampung and Java, *An. sinensis* in area of Jambi, *An. kochi* in Sulawesi and *An. barbirostris* in region of East Nusa Tenggara. There are several species of Anopheles mosquitoes, which need to be controlled in Lahat such as *An. nigerimus* and *An. letifer* (that are confirmed as malaria vectors) in South Sumatra, *An. aconitus* and *An. sinensis* (that are confirmed in border of South Sumatra and Lampung Provinces). It relates with a high population mobility among this three provinces.

Man hour density (MHD) can be seen in table 1, which showed 0.02 mosquitoes/person/hour and the highest MHD was 0.92 per person per hour. Species Anopheles with highest man biting rate (MBR) was *An. vagus*, consisted of 7.33 in Lebak Budi village, while the highest MBR reached 90.11. High density of vector populations can increase contact a vector that infective towards human [8]. Certain mosquito density is one of the conditions for becoming a vector along with other terms such as the age of mosquitoes, there is contact with human beings, susceptible to the parasite and there is the source of the transmission [5].

An. vagus caught almost in all regions in Lahat. *An. vagus* is also confirmed as malaria vector in some other areas in Indonesia, such as Kulon Progo [9] and Sukabumi [10]. In Bunga Mas village, none of mosquito Anopheles spp were caught. Catching of mosquito has attempted to close to breeding places a day before the survey was conducted, and a high larvae density were found in puddle with 10 n^2 size. The bite of an infected anopheles mosquito spp can be seen on figure 1.

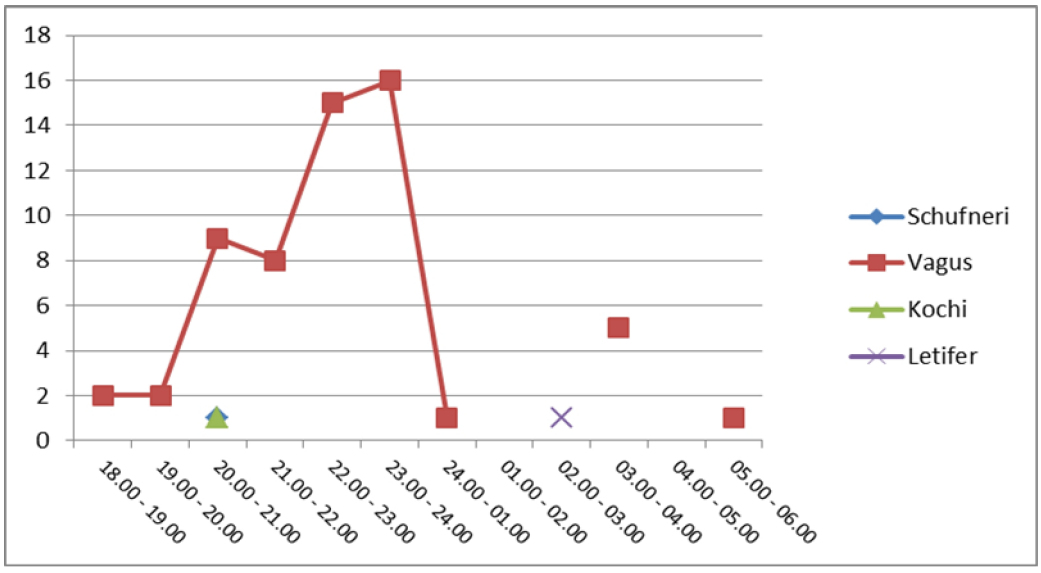


Figure 1. Peak of bite activity of *An schufneri, An. vagus, An. kochi* and *An. letifer*

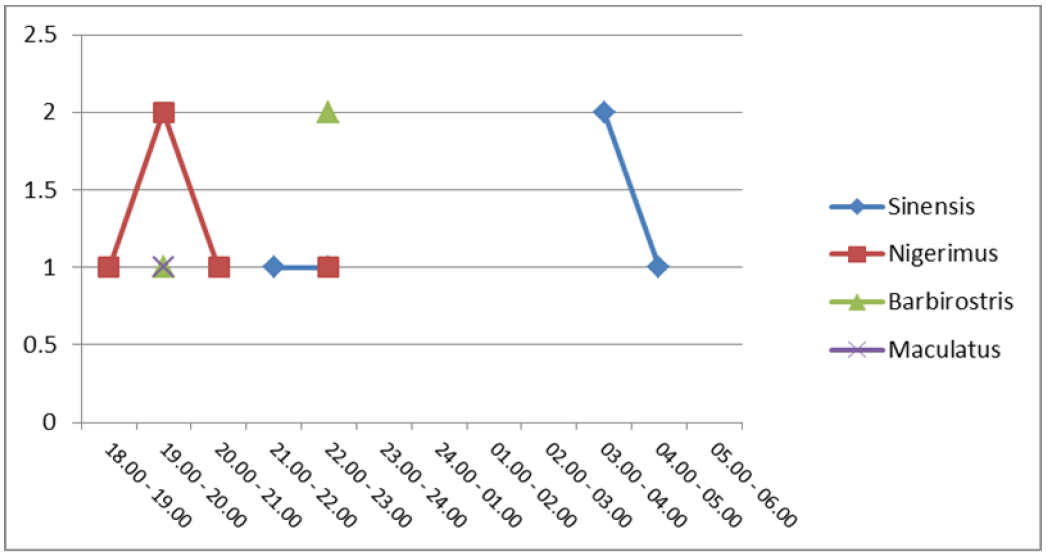


Figure 2. Peak of bite activity of *An. sinensis, An. nigerimus, An. barbirostris* and *An. maculatus*

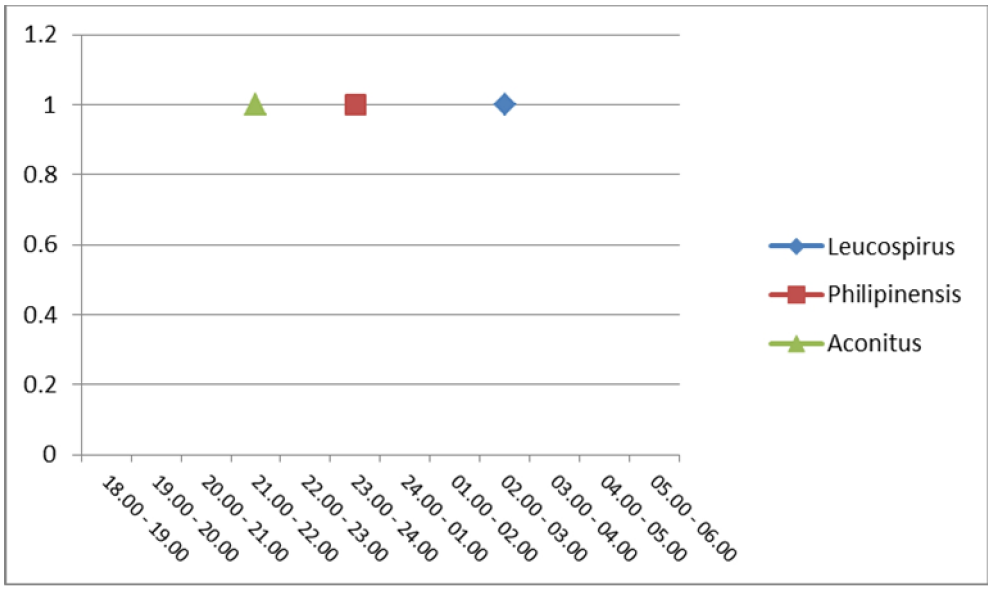


Figure 3. Peak of bite activity of *An. leucospirus*, *An. philipinensis* and *An. aconitus*

From Figure 4 it looks that *An. nigerimus* is found in almost every location of the arrests. *An nigerimus* has been confirmed as vectors of malaria in South Sumatra Province. With the vectors of malaria is distributed throughout a region almost Lahat district will encourage the high number of malaria cases.

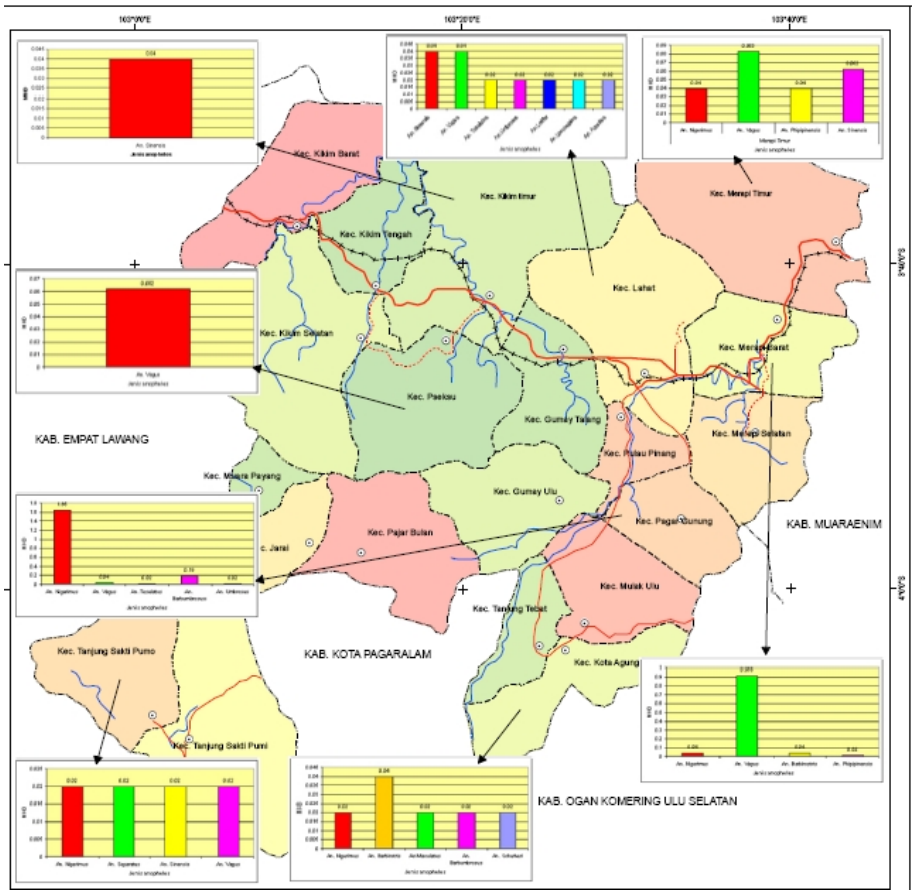


Figure 4.Man hour density

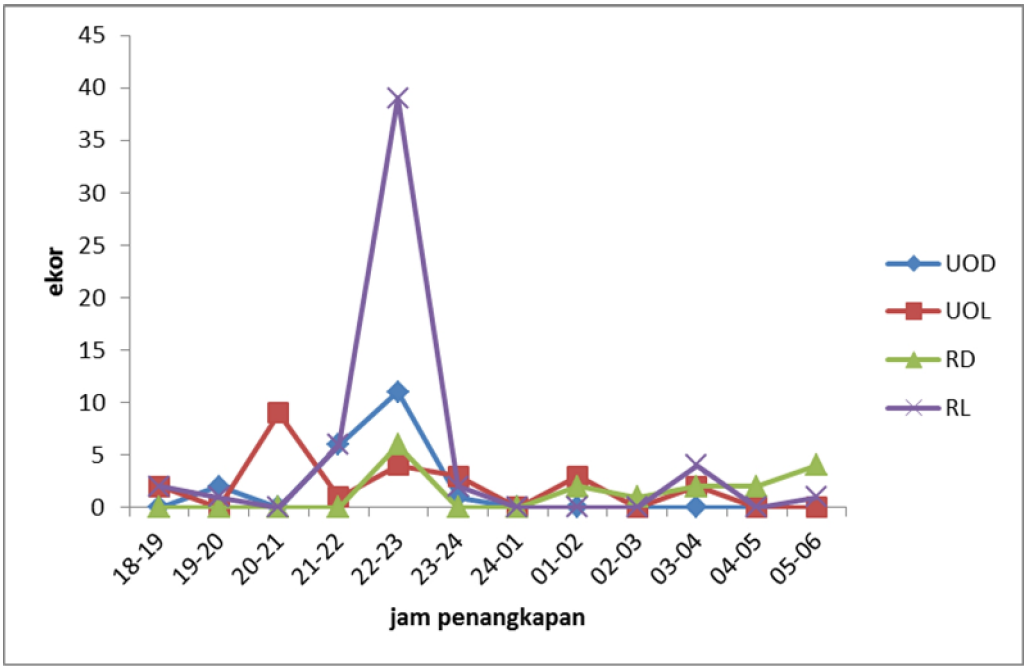


Figure 5. Arrest mosquito Anopheles results with several methods of arrest

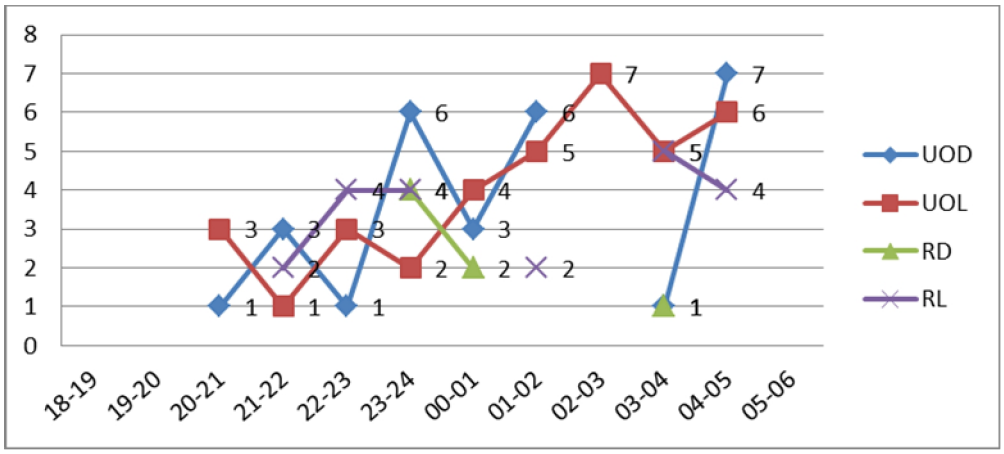


Figure 6. Arrest mosquito Anopheles results with several methods of arrest

Anopheles in Lahat district showed exophagic characteristics, which is more like outdoor biting rather than indoor). Increasing contact with infected Anopheles mosquito especially of vectors of malaria mosquitoes with human beings can also be caused by disruption of the environment because the forest due to the opening of new land that causes a rise in the incidence of malaria. At Lahat opening land-new land during the last few years quite popular recently earmarked as the area of plantations (oil palm, rubber) as well as the area of coal mine. Research conducted in Malaysia said that the opening of a forest that is used as land palm plantations significantly impact on the occurrence of a decrease in abundance of four species of an infected anopheles, but *An. letifer* not show the changes of abundance [12].

An. letifer in our study preferred to rest outdoor (exophilic). According to breeding place, vectors of malaria can be grouped in three types namely breed in the riverbanks, hills/woods and beach/river. Vectors of malaria that breed in the riverbanks is *An. aconitus, An. annullaris, An. barbirostris, An. kochi, An karwari, An. nigerrimus, An. sinensis, An. tesellatus, An. vagus, An. letifer*. Vectors of malaria that breed in hills/forests are *An. balabacensis, An. bancrofti, An. punculatus, An. umbrosus*. As for the coastal regions/riversides the vectors are *An. flavirostris, An. koliensis, An. ludlowi, An. minimus, An. punctulatus, An. parangensis, An. sundaicus, An. subpictus* [2]. If it is assumed that transmission of malaria occurring in the village of (rather than imports); then the forest, environmental factor of a like kind the cultivation, precipitation and proximity of a river or other body of water constituting the parameters that are useful to identify the existence of vectors and its role in communicability malaria [11].

Malaria vector biting activity time is known, 17.00-18.00, 24 hours before (20.00-23.00), after 24 hours (00: 00-4.00). The malaria vector activity peak in 17.00-18.00 is *An. tesselatus*, 24 hours before is *An. aconitus, An. annullaris, An. barbirostris, An. kochi, An. sinensis, An. vagus*, while the 24 hours after the bite is *An. farauti, An. koliensis, An. leucosphyrosis*, and *An. punctullatus* [2]. Behavior vector malaria as breeding place and time activity bite is very important known by the decision makers as a basis consideration to determine effective intervention in control a vector.

ELISA test concluded that all samples (240 tail) were spotozoite negative. This condition possibility caused by the low circulation a Plasmodium outstanding on

mosquitoes vector (not peak of transmission) or because of the lack the number of mosquito that tested.

CONCLUSION

An. nigerimus that are vectors of malaria in South Sumatera Province, also found distributed in several areas of Lahat. The need for support of local government and the public related to the prevention of the bites of *An. nigerimus* that are vectors of malaria in the region of south sumatra province so that it can reduce unemployment in pain and malarial deaths.

REFERENCES

1. Depkes RI, " Kep. Menkes RI tentang Eliminasi Malaria di Indonesia", Dirjen PP&PL 2009.
2. Kemenkes RI, "Buletin Jendela Data dan Informasi Kesehatan, Tri Wulan I, 2011.
3. Laporan pengelola program malaria Propinsi Sumatera Selatan tahun 2011.
4. Laporan Pengelola Program Malaria Dinkes Kabupaten lahat.
5. Depkes RI, "Pedoman ekologi dan aspek perilaku vektor", Ditjen PPM & PL, 2001.
6. Reid, JA, Anopheline Mosquitoes of Malaya and Borneo. Studies from the Institute of Medical Research of Malaysia. Bulletin No 31, 1968.
7. Bogh, C, Malaria in the Coffee Gardens of South Sumatera (Summary of Findings from Surveys Done by OKU-VBDC). Intensified Communicable Disease Control Project.
8. Mc.Kelvey JJ, Eldridge BE, Maramorosch K. Vector of Disease Agents Interaction with Plants, Animal and Man. New York: Praeger Publisheres, CBC Educational and Professional Publising a division of CBC, Inc. 521 Fith Avenue. 1991.
9. Wigati, RA, Mardiana, Mujiyono dan Alfiah S. Deteksi Protein Circum Sporozoite Pada Spesies Nyamuk Anopheles vagus Tersangka Vektor Malaria di Kecamatan Kokap, Kabupaten Kulon Progo Dengan Uji Enzyme Linked Immunosorbent Assay (ELISA). *Media Litbang Kesehatan* Volume XX No 3, 2010: 118-123
10. Munif A, Rusmiarto S, Aryati Y, Andris H, dan Stoops CA. Konfirmasi Status Anopheles vagus Sebagai Vektor Pendamping Saat Kejadian Luar Biasa Malaria di Kabupaten Sukabumi Indonesia. Jurnal Ekologi Kesehatan Vol.7 No.1, 2008: 689-696.
11. Hakre S, Masuoka P, Vanzie E, Roberts DR. Spatial correlations of mapped malaria rates with environmental factors in Belize, Central America. *Int J Health Geogr* 2004; 3: 6.
12. Chang MS, Hii J, Buttner P, Mansoor F. Changes in abundance and behaviour of vector mosquitoes induced by land use during the development of an oil palm plantation in Sarawak. *Transac. Roy. Soc. Med. Hyg.* 91(4), 1997;382-386.

VERTICAL TRANSMISSION OF DENGUE VIRUS IN *Aedes aegypti* MOSQUITO

Devita Febriani Putri[1,2]

[1]Faculty of Medicine, Malahayati University, Lampung
[2]Ph.D Student, Faculty of Medicine, Gadjah Mada University, Yogyakarta
Correspondence: devita.putri@gmail.com; devita_febriani@yahoo.com

ABSTRACT

Background: Dengue infection is transmitted to humans by Aedes (Stegomyia) mosquitoes, with is *Aedes aegypti* as the principal vector. Dengue infection has been incriminating in major dengue outbreaks worldwide.
Results: The existence of dengue virus is through horizontal and vertical transmission. The best documented mechanism is dengue virus with horizontal transmission, which maintained by human-aedes mosquito-human cycle. However, vertical or transovarial transmission, where the female-infected mosquito is able to transmit the virus to their progeny, has been reported experimentally. In nature, several studies have confirmed vertical or transovarial transmission of dengue virus in infected *Ae. aegypti* larvae and from adult reared from them or from wild-caught adult mosquitoes.
Conclusion: Vertical transmission of dengue virus in *Ae. aegypti* were previously not considered to contribute to epidemiology of dengue transmission. Now, the vertical transmission evidently played important role in initiating and maintaining in dengue epidemic. More study is needed to supporting factor in predicting dengue outbreaks and more effective control of dengue.
Keywords: dengue virus, *Ae. aegypti*, vertical transmission.

BACKGROUND

Dengue is mosquito-borne viral disease in human which distributed in the tropical and subtropical regions of the world. Dengue infection is cause by dengue (DEN) virus with 4 serotype (DEN1, DEN2, DEN3, DEN4), of the genus Flavivirus. They are transmitted to humans by Aedes (Stegomiyia) mosquitoes, which are *Aedes aegypti* is the principal vector, and has been incriminated in major dengue outbreaks worldwide [1,2].The first reported of epidemics of dengue fever occurred in 1779-1780 in Asia, Afrika and North America. After that, dengue in Australia noted in 1897. The global epidemiology and transmission dynamic of dengue virus were changed dramatically in Southeast Asia after World War II. The change of ecology caused by war expanded the geographical distributionn of *Ae. aegypti* and making many country in this region very permissive for epidemic dengue transmission [3].

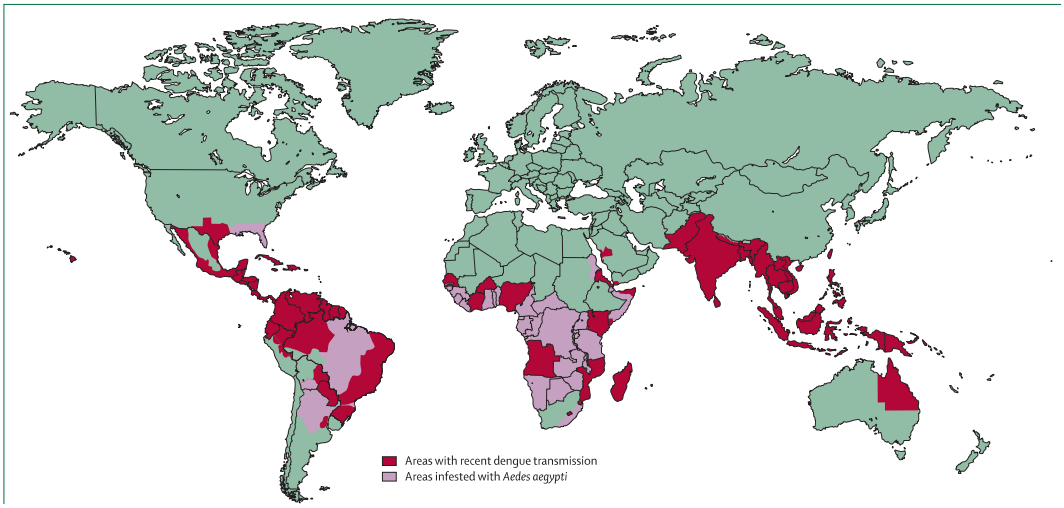


Figure 1. Approximate global distribution of dengue and *Aedes aegypti* in 2005.

In Indonesia, the first case report of Dengue Hemorrhagic Fever (DHF) was from Surabaya and Jakarta in 1968. Since then, DHF was distributed throughout Indonesia. The rate of incidence has increase each year, even morbidity and mortality. DHF outbreaks initially occurs every five years, and then became every 3 years, then changed to 2 years, and finnaly 1 year. Several possible causes that are demographic and societal changes such as population growth, urbanization, modern transportation, climate change and the relative humidity, and increasing mosquito breeding places specially in slum areas in certain seasons [4].

The existence of DEN virus in nature is through horizontal and vertical transmission. The best documented mechanism is dengue virus with horizontal transmission, which maintained by human-aedes mosquito-human cycle. However, vertical or transovarial transmission, where the female-infected mosquito is able to transmit the virus to their progeny, has been reported experimentally [5,6]. In nature, several studies have confirmed vertical or transovarial transmission of dengue virus in infected *Ae. aegypti* larvae and from adult reared from them or from wild-caught adult mosquitoes [7-14]. The mosquito plays an important role for DEN virus transmissionand maintaining the presence of DEN virus, especially where the circumstances unfavorable in nature [2].

RESULT

Vertical transmission is transference of infectious agent from a male and female parent to its progeny [1]. According to Leake [15], there are 3 mechanisms of arbovirus vertical transmission in mosquitoes as follow: 1. Uninfected female mosquitoes suck blood viremik host, the virus replicates in the mosquito and infected eggs produce larvae that infectious; 2. Uninfected female mosquitoes mate with infected males transovarial and mosquito mating occurs during sexual transmission (venereal transmission), and as a result the female ovaries infected with a virus; 3. Female mosquito ovaries network virus infection and maintained up to the next generation genetically.

Vertical transmission in wild mosquitoes population

Figure 2. Distribution study of vertical transmission dengue virus in wild *Aedes aegypti* population

Vertical transmission in natural populations of their hosts has been reported for DENV. In Rangoon, Myanmar, the following minimum infection rate (MIR) was determined for *Ae. aegypti* adult males reared from wild-caught larvae, 0.26 and adult females, 0 [7]. In Subcontinent India, only females were infected, and no DENV was recovered during the period May to December 1993 (n = 164) [8]. In Tamil Tandu, Southern India, transovarial DENV in *Ae. aegypti* was detected from larvae at November 1997, January-March 1997 with mean monthly temperature: min 16-24°C, max 26-39 °C [9].

In Thailand, transovarial transmission DENV 2 in *Ae. aegypti* was determined from progeny of infected mosquitoes in third generation [10]. In Malaysia, MIR for *Ae. aegypti* larvae ranged between 5.77-40. When dengue occurrences were analysed together with transovarial transmission of DENV in mosquito, it was found that transovarial DENV often occurred prior to the reporting of human cases. The intervals between detecting the transovarial DENV and the occurrence of first clinical case/human case ranged from 7 to 41 days [11].

In The State of Oaxaca, Mexico, DENV 2, DENV 3, and DENV 4 were detected in 2 pools *Ae. aegypti* (43 pools of in cage born mosquitoes) [12]. In Rajasthan India, there is not active transovarialtransmission DENV in *Ae. aegypti* during winter season [13]. In Tamil Tandu India, MIR among adult males was high in June 2003 (0.028), there was 3 positif pool for DENV 2 (2 pools), and DENV 3 (1 pool) [14].

The first study of vertical transmission in *Ae. aegypti* in Indonesia was performed by Umniyati [16] in Klitren of Yogyakarta using method of Immunocytochemistry Peroxsidase Streptavidin Biotin Complex (ISBPC) on the head squash mosquitoes with transovarial Infection Rate (TIR) of 27.27%. Mardihusodo *et al*. study [17] in Yogyakarta showed precentage of transovarial transmission DENV in *Ae. aegypti* wass 38.5-70.2 %. In Bandung, transovarial transmission DENV 2 was detected in *Ae. aegypti* using PCR [18]. Widiarti *et al*. [19] proved that the population of *Ae . aegypti*

from 6 district of 5 endemic area in Central Java Province, have shown the transovarial transmission of DENV. Hartanti *et al*. (2010)demonstrated the occurrence of transovarial transmission DENV in *Ae. aegypti* in Tebet, Jakarta.

Experimental studies with dengue virus.

The results of laboratory experiments on vertical transmission should be interpreted carefully. Many experiments, start with intrathoracic inoculation of an virus into adult female mosquitoes, to be followed some days later with ingestion of an uninfected blood meal. This procedure bypasses both the midgut-infection and midgut-escape barriers and rapidly leads to a disseminated infection As noted later, vertical transmission tothe progeny of orally infected females generally does not occur in the gonotrophic cycle in which the females become infected, but in the next and any subsequent gonotrophic cycles [20].

The second theory of Leake [15] about venereal transmission was proved by Rosen [21]. That venereal transmission of virus, from males to females, can lead to vertical transmission was shown experimentally. Male *Ae. albopictus* were parenteraly infected with DENV-1, and virgin females were given a non-infective blood meal. The mosquitoes mated 14 days after the males had been infected and 3 days after the females had taken their blood meals, at which time their oocytes were mature and enclosed in an impermeable chorion. No eggs laid during the period 0–72 hour after mating produced infected larva (Table 1). The first infected eggs were laid 73–96 hour after mating, is after an interval long enough for viral replication in the female reproductive tract, and thereafter the minimum infection rates increased.

Table 1. Infection of F1 progeny following venereal transmission of DENV-1 from male to females of *Aedes albopictus* by time between mating (when the females became infected) and oviposition.

Hours after mating eggs laid	Number F_1 larvae tested	Pools positive/ pools tested	Minimum infection rate
<24	86	0/11	
25–48	2,456	0/39	
49–72	7,756	0/80	
73–96	6,968	1/74	1:6,968
97–120	3,129	4/33	1:782
121–144	1,208	4/16	1:302
145–264	850	4/38	1:213

From his experimental findings, Rosen [5] postulated that, when female mosquitoes have been venerealy infected with a flavivirus, virions that have accumulated in the reproductive tract invade some oocytes at the time of ovulation, just before oviposition. He further postulated that that is the mechanism of vertical infection by DENV generally. In contrast to transovarian transmission, such transovum transmission permits the infection of progeny after a single maternal blood meal, especially if oviposition is delayed.

The vertical transmission of DENV from parenteraly infected females of *Aedes sp* to their F_1 progeny was low, but that from vertically infected F_1 females to their progeny was higher. Thus, in one study in *Ae. aegypti* when females of the parental generation were inoculated with DENV-3; the vertical-transmission rates were at percentage of F_1= 0.028; F_2- F_7=0.086 to 0.126 as shown in table 2 [6].

Tabel 2. Persentage of mosquitoes positive for dengue virus antigen in successive generation of *Aedes aegypti* mosquitoes.

	Males		Females		
	Tested	Number positive (%)	Tested	Number positive (%)	Total Percent positive
F_1	123	3 (2.4)	167	5 (3.0)	2.8
F_2	158	12 (7.6)	144	14 (9.7)	8.6
F_3	258	22 (8.5)	173	34 (19.7)	13.0
F_4	122	8 (6.6)	125	21 (16.8)	11.7
F_5	154	14 (9.1)	166	23 (13.9)	11.6
F_6	145	13 (9.0)	120	18 (15.0)	11.7
F_7	157	16 (10.2)	176	26 (14.8)	12.6

CONCLUSION

The effective vertical transmission rates for a host virus combination may be higher in the laboratory than in the field, where females must survive natural hazards to complete a second gonotrophic cycle.The probable mechanism of transovarial transmission is entry of virions through the oocyte during ovulation (so called transovum transmission), but how this occurs is not clear. During the secondand later gonotrophic cycles, invasion of oocytes by residual virus may be possible. Once vertical infection has occurred, by whatever means, transovarial transmission to the following generation should be possible.

Vertical transmission of dengue virus in *Ae. aegypti* were previously not considered to contribute to epidemiology of dengue transmission, evidently played important role in initiating and maintaining in dengue epidemic. Mosquito can act as reservoirs for DENV, because vertical transmission can detect up to seventh generation. More study is needed to supporting factor in predicting dengue outbreaks and more effective control of dengue.

REFERENCE:

1. WHO, SEARO. 2003. Pencegahan dan Penanggulangan Penyakit Demam Dengue dan Demam Berdarah Dengue, Kemenkes RI. Jakarta.
2. Mardihusodo, S.J. 2011. Dengue Virus Transovarial Transmission In *Aedes aegypti* L: Potential To DHF epidemics. *Seminar Nasional Penyakit-Penyakit Infeksi dan Vektor*. Universitas Malahayati, Lampung. 15-17 Desember 2011.
3. Gubler, D.J. 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Micro*. 10.
4. Soegijanto, S. 2008. Bahaya yang Mengintai Endemisitas DBD di Indonesia. *Demam Berdarah Dengue*. Edisi 2. Airlangga University Press. Surabaya. 25-44.

5. Rosen, L. 1988. Further Observation on The Mechanism of Vertical Transmission of Flaviviruses by Aedes Mosquitoes. *Am J Trop Med Hyg.*39(1) : 123-26.
6. Joshi, V., Mourya D.T., Sharma, R.C. 2002. Persistence of Dengue-3 Virus Through Transovarial Transmission Passage in Successive Generations of *Aedes aegypti* Mosquitoes. *Am J Trop Med. Hyg.* 67: 158-161.
7. Khin, M.M., Than, K.A. 1983. Transovarial Transmission of Dengue 2 Virus by *Aedes aegypti* in Nature. *Am J Trop Med. Hyg.* 32:590-4.
8. Joshi, V., Singhi, M., Chaudhary, R.C. 1996. Transovarial transmission of dengue 3 virus by Aedes aegypti. *Short report Transaction of The Royal Society of Tropical Medicine and Hygyiene*. 90: 643-644.
9. Thenmozhi, V., Tewari, S.C., Manavalan, R., Balasubramanian, A., Gajanana, A. 2000. Natural Vertical Transmission of Dengue Viruses in *Aedes aegypti* in Southern India. *Trans of Roy Soc Trop Med Hyg*. 94:507.
10. Wasinpiyamongkol, L., Thongrungkiat, S., Jirakanjanakit N., Apiwathnasorn, C. 2003. Susceptibility and Transovarial Transmission of Dengue Virus in *Aedes aegypti*: Preliminary Study of Morhological Variation.
11. Lee, H.K, Rohani, A. 2005. Transovarial Transmission of Dengue Virus in *Aedes aegypti* and *Aedes albopictus* in Relation to Dengue Outbreak in Urban Area in Malaysia. *Dengue Bull. WHO*. 10:106-11.
12. Gunther,D.J., Munoz, J.P.M., Ishiwara, D.G.P., Benito J.S. 2007. Evidence of Vertical Transmission of Dengue Virus in Two Endemic Localities in the Sate of Oaxaca Mexico. Intervirology. 50:374-52.
13. Angel, B., Joshi, V. 2008. Distribution and Seasonality of Vertical Transmitted Dengue Viruses in Aedes Mosquitoes in Arid and Semi-Arid Areas of Rajasthan, India. *J Vector Borne Dis*. 48: 56-9.
14. Arunachalam, N., Tewari, S.C., Thenmozhi, V., Rajendran, R., Paramasivan, R., Ayanar, K., Tyagi, B.K.2008. Natural Vertical Transmission of Dengue Viruses by *Aedes aegypti* in Cennai, Tamil, India. *Indian J Med Res* 2008. 127: 395-7.
15. Leake, C.J. 1984. Transovarial Transmission of Arbovirus by Mosquitoes. In MA. Mayo and K.A Harrap (eds) Vector in Virus Biology, 197(33): 159-74.
16. Unmiyati, S.R. 2004. Preliminary Investigation On The Transovarial Transmission of Dengue Virus in The Populatian of *Aedes aegypti* in the Well. *Seminar Peringatan Hari Nyamuk IV*. Surabaya
17. Mardihusodo SJ, Satoto TBT, Mulyaningsih B. Umniyati SR. & Ernaningsih. Bukti adanya Penularan Virus Dengue Secara Transovarial Pada Nyamuk Aedes aegypti di Kota Yogyakarta. *Simposium Nasional Aspek Biologi Molekuler, Patogenesis, Manajemen dan Pencegahan KLB*. UGM, Yogyakarta. 16 Mei 2007.
18. Akbar, M.R., Agoes, R., Djatie T., Kodyat, S. 2008. PCR Detection of Dengue Transovarial Transmissibility in *Aedes aegypti* in Bandung, Indonesia. *Proc ASEAN Congr Trop Med Parasitol*.3:84-9.
19. Widiarti, Boewono, D.T., Widyastuti. 2009. Deteksi Antigen Virus Dengue pada Progeni Vektor Demam Berdarah dengan Metode Imunohistokimia. *Bul Penelitian dan Kesehatan*. 37 : 126-36.
20. Clement. 2010. Arboviruses – characteristic and concepts. *Biology of Mosquitoes*. Volume 3. Cambrige. 90 -162.
21. Rosen, L. 1987. Sexual Transmission od Dengue Viruses By *Aedes Albopictus*. *Am J Trop Med Hyg.,* 37(2):398-402.

INTEGRATED VECTOR MANAGEMENT IMPLEMENTATION IN CIMAHI: A CASE STUDY

M. Ezza Azmi Fuadiyah, Roy Nusa RES
Research and Development Unit on Animal-Borne Diseases Control, Ciamis
Correspondence: ezzaazmi@gmail.com

ABSTRACT

Background: Cimahi city has higher incidence of dengue hemorrhagic fever (DHF) since 2004. In 2008, the case tended to decrease. This study aimed to explain the application of integrated vector management in Cimahi city to increase DHF incidence.
Method: This was a literature review of studies on DHF in Cimahi city in 2010
Result: Cimahi city succeed to decrease DHF incidence as the city applied integrated vector management, along with community empowerment and participation.
Conclusion: Application of integrated vector management and community participation may assist the program of DHF control.
Keywords: dengue hemorrhagic fever, integrated vector management

BACKGROUND

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus that transmitted through *Aedes aegypti* or *Ae. albopictus* bite. The number of DHF cases in Indonesia tends to increase and the spread become wider by the year [1]. West Java province is one of the region with high number of DHF cases. The number of DHF patients and the Incidence Rate (IR) of DHF in West Java are always increasing since 2000 until 2009. The IR of West Java Province in 2000 is 13, 8/100.000 and by 2009 has reached 89, 41/100.000. In 2007, the entire region in West Java Province was reporting an outbreak of DHF. The year after the outbreak, DHF case number was indeed decreasing but still higher than the number of cases before the outbreak and increase by the year [2].

Cimahi with its nearest neighbor region that are Bandung, Kabupaten Bandung and Kabupaten Bandung Barat are geographically similar and all of them are the regions with highest DHF IR in West Java. Graphic 1 shows while the other three have a slight decrease in IR, the city of Cimahi has a bigger decrease on IR in 2010 than the year before. The decrease in DHF cases were, of course, not only because of natural causes but also perhaps because the effort the city made in controlling the vector of DHF [2]. Assuming with a relatively fixed population, there is a bigger decrease of IR in Cimahi than its neighbor region. It could be the result of DHF control in Cimahi.

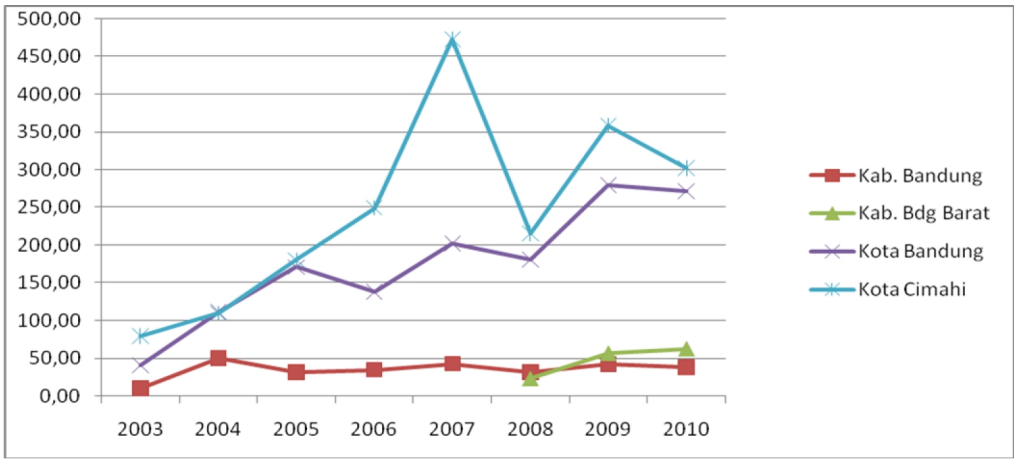
Integrated Vector Management (IVM) is a rationally decision making process in optimal vector control with five important elements which are evidence based decision making; integrated approach, collaboration within health sector and with other sectors; advocacy, social mobilization and legislacy; and capacity building [3].

METHOD

This was a qualitative study to construct reality and understanding its meaning, with paying attention to the process, the event and the authenticity as the key about study object[4]. Primary data was obtained through in-depth interview with purposive sampling method and snow ball. Secondary data was obtained from case report and through related document review. The base of document using for research, as Guba and Lincoln statement in Basrowi, is the documents as stable data resource and can be used to proof an experiment [5]. Documents that were used in this study consisted of document on DHF control strategy, city regulation related to DHF control, along with official government statement related to DHF control. Samples of document were chosen using non-probability sampling method where the documents chosen by the purpose of the study.

The documents were pre-analyzed where it's been read, seen through, and completed. After that, the documents were classified in categories. Document's analysis was done using chronologically causal relationship between the activities found in documents with DHF cases. Interpreting documents were done by making quotation than change it into text, labeled and categorized. The result of interview and documents review describe about DFF control method were done in Cimahi. That result then explained using IVM strategy approach.

RESULT



Graphic 1. Comparison of dengue hemorrhagic fever incince rate between Cimahi and its neighbor region

Cimahi was experiencing a DHF outbreak in 2007 and continually had high DHF IR until 2009. Cimahi government through regulation (stated by Cimahi mayor) established a DHF control strategy. The regulation was effectively used in 2010. The strategy was done by involving entire sector in government and community. DHF control in Cimahi was a government program that obliges all the government office to participate with Health Office as the supervisor. Some of the actions included in DHF control strategy were preventive actions such as serial larva inspection (*pemeriksaan jentik berkala*/PJB) by the community and elementary students, health promotion

through various channels, and vector control actions such as larvicides using and selective fogging.

PJB

Breeding place eradication (*Pemberantasan Sarang Nyamuk*/PSN) as a vector control method was recommended to hold by community using PJB as the evaluation instrument. PJB was done by local larvae inspectors (*Juru Pemantau Jentik/Jumantik*) which usually are health cadres from local housewives. *Jumantik* were expected to report their inspection result weekly to local district government that will passed it to Health Center (*Puskesmas*). PJB was also done by elementary students (grade 4 and 5). They expected to inspect larvae existence in their own houses and five other houses in their neighborhood and then report it to their teacher. The teacher will deliver it to *Puskesmas*.

All other government offices of Cimahi besides Health office were responsible to certain district. If there was a case found or increase on entomology index of DHF vector in a district, the government office responsible for the district, and Health Office as supervisor will reprimanded by the city mayor. The mayor and the entire government officials involved in larvae inspection periodically in random district.

There were few obstacles in the PJB activities that came from community as target population or from *Jumantik* as the officer. *Jumantik* had responsibility to routinally perform larva inpection. The routine was expected to push the community to do PSN regularly at least before the inspection. Obstacle from community usually in their reluctant to allow *Jumantik* enter their houses. Obstacle from *Jumantik* was boredom as the inspection must be done every week. The boredom of *Jumantik* could lead to inaccuracy data.

Health promotion

Health promotion was held by disseminating information about DHF and its vector control in places and events that involving community member such as in school, religious meeting, regular community meeting, and district regular meeting. Besides that, information also spread through some community empowerment initiated by Health Office in 2010 such as DHF related knowledge contest, debate contest and DHF control innovation contest. These events were held to find out how deep the community knowledge about DHF control related matters and to create an alternative idea about DHF control method that can be done by community themselves.

Selective larvicides using

Cimahi Health Office distributed temephos as larvicide through *puskesmas'* to be used by the selective community area in public water reservoir or water storage that difficult to periodically clean. In the subdistrict of Melong there was a huge water container that potential to be vector breeding place so it's recommended to dry, clean and refill it before using temephos. The community was also trying to reduce the existence of potential breeding places by using temporal water storage in their bathroom so they can dump the excess water and dry it after using.

Fogging (indoor and outdoor spraying)

Fogging (ultra low volume spraying) is a vector control method targeted to adult mosquitos using insecticide [6]. There were two types of fogging in Cimahi, mass and focus fogging. Mass fogging was usually held in larger area, while focus fogging in DHF patient neighborhood.

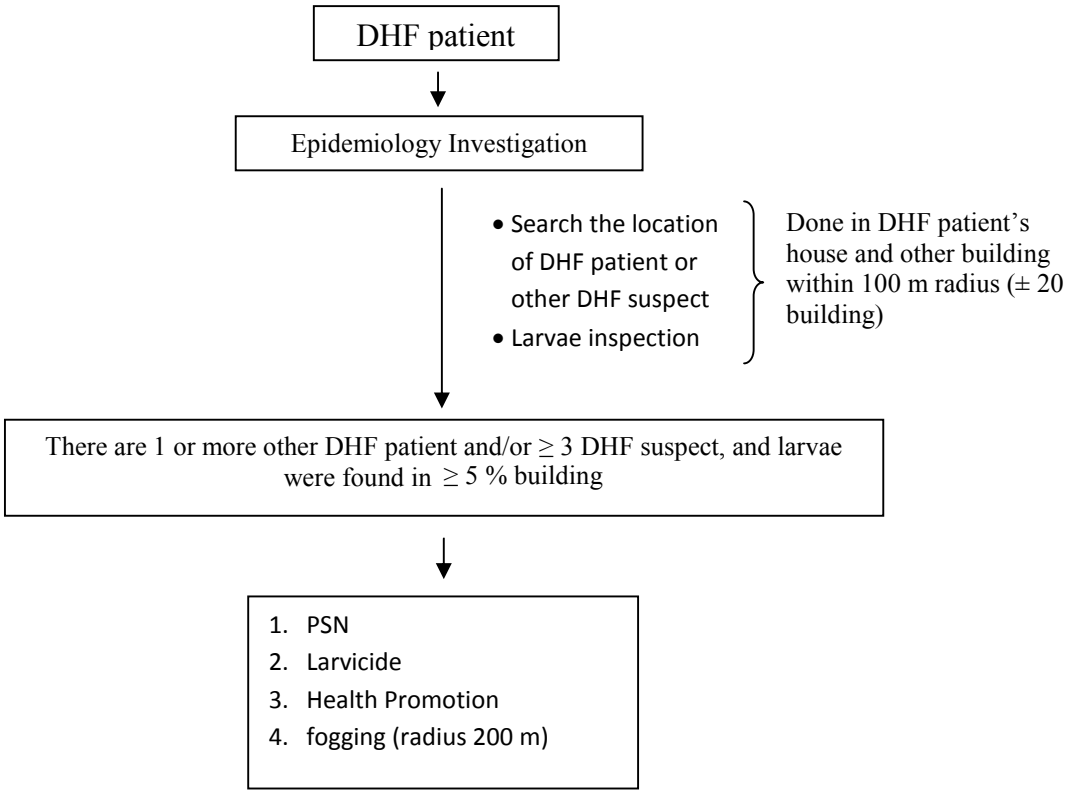


Figure 1.The Sequence of DHF Patient Field Treatment

Figure 1 showed steps of fogging in Cimahi that was done by following the DHF patient field treatment sequence [6]. Fogging will only be done if epidemiology investigation showed 1 or more other DHF patients, and/or ≥ 3 DHF suspect in neighborhood, and larvae were found in ≥ 5 % building. This also confirmed that fogging was the last step after PSN, using larvicide and health promotion to the community in the neighborhood.

Cimahi Health Office used two kinds of insecticides in 2010, cypermethrin and malathion. Those insecticides were sent by West Java Province Health Office, which obtained it from Ministry of Health. The devices they use were swing fog machine with varying brand obtained, also from West Java Province Health Office.

DHF cases monitoring

DHF cases were daily reported by '*puskesmas'* and hospitals to Cimahi Health Office. All hospitals, either public or private, must report DHF. The hospitals consisted of Mitra Kasih Hospital, Mal Hospital and Dustira Hospital in Cimahi, also Dr. Hasan Sadikin Center Hospital, Rajawali Hospital, Kebonjati Hospital, and Hermina Maternal

and Child Hospital in Bandung. They reported DHF cases to Cimahi Health Office using facsimile or email.

DISCUSSION

DHF is one of the vector borne disease with *Aedes aegypti* and *Ae. albopictus* as its vector so this disease control is more depend on vector control. Vector control is any activity and action done in purpose to reduce vector population so the existence of it would not be a risk for transmission of vector borne disease happened in certain area or way to avoid contact between people and vector so the transmission can be prevented [7].

Vector control can be done in many ways or approach such as through environmental control such as environmental modification or manipulation. The other ways include methods such as physic/mechanic, biology or chemical control approach. Vector control also can be done by not using vector as target but the people which by change their behavior related to vector control [6]. However, any methods would not be guaranteed to succeed if applied separately and without any prior assessments. A new approach was needed to revive vector control. Such an approach was to resolve not only technical issues pertainingto vector control implementation, but also, to take into consideration health systems constraints as well as environmental concerns related to the judicious use of insecticides [6].

Since 2001 the World Health Organization has been promoting Integrated Vector Management (IVM) as the new strategic approach to vector control [8]. IVM is defined as the targeted use of different vectorcontrol methods alone or in combination to preventor reduce human-vector contact cost-effectively, whileaddressing sustainability issues.

Figure 2. Integrated Vector Management Framework [10]

Two points need to be emphasized, first; IVM is based on the premise that effective control is not the sole preserve of the health sector but requires the collaboration of various public and private agencies and the participation of the

communities. Secondly, IVM emphasizes capacity building at the district and municipal level to plan, implement, monitor and evaluate these vector control operations [9].

IVM is a rationally decision making process in optimal vector control with five important elements which are evidence based decision making; integrated approach, collaboration within health sector and with other sectors; advocacy, social mobilization and legislacy; and capacity building [10].

1. **Evidence based decision making**. Adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation.
2. **Integrated approach**. Ensure rational use of available resources through a multi-disease control approach, integration of non-chemical and chemical vector control methods, and integration with other disease control measures, such as active and passive case detection and treatment.
3. **Collaboration within health sector and with other sector**. Consideration of all options for collaborationwithin and between public and private sectors; strengtheningchannels of communication among policymakers,vector-borne disease control programme managers andother IVM partners.
4. **Advocacy, social mobilization, legislacy**. Promotionand embedding of IVM principles in the developmentpolicies of all relevant agencies, organizations andcivil society; establishment or strengthening of regulatoryand legislative controls for public health and pesticidemanagement; empowerment of communities.
5. **Capacity building**. Development of essential physical infrastructure, financial resources and adequate human resources at local and national levels to manage IVM programmes based on needs assessments

Implementation of DHF control strategy in Cimahi since 2000 also based on IVM strategy above, using its five important elements.

1. **Evidence based decision making.** The decision making of DHF control strategy was based on (1) DHF Case Report that obtained from daily report by Community Health Center (Puskesmas) and Hospital, (2) Report of Epidemiology Investigation held in DHF patient's neighborhood, (3) The report of vector entomology index, and (4) Any research report that related to DHF and its vector control. The government budget and other resources also become an input for decision making related to DHF control strategy.
2. **Integrated approach.** DHF vector control in Cimahi was conducted by using several methods that are (1) Chemical approach using temephos for selective area larva control and cypermethrin&malathionas insecticides used in fogging with spraying it in house and neighborhood of a selective DHF patient, (2) Environment modification by manipulating potential breeding places so the vector can't breed comfortly. It was done with Vector Breeding Place Eradication (PSN) which it's result evaluated using Serial Larvae Inspection (PemeriksaanJentikBerkala/PJB), (3) Using temporary water container in bathroom so it will be easier to clean and minimalize the existence of potential breeding place, (4) Case monitoring actively held with the daily report of the case from Puskesmas and Hospitals, (5) Epidemiology Investigation was held immediately by Puskesmas officer soon after they got the report from Cimahi Health Office.

3. **Collaboration within health sector and with other sector.** Health Office of Cimahi is working together with public and private hospitals in Cimahi and in the nearest city, Bandung, to monitoring DHF cases. The other government offices have responsibility in DHF vector control in certain district in the city and will get reprimand from the mayor if the entomology index or the cases in the district is increase.
4. **Advocacy, social mobilization, legislacy.** Disseminations about DHF related information was done through many channels like government official statements, the city government monthly meeting, hearing with the legislative council, social forums such as Pengajian, serial community meeting. Social mobilization was done through community empowerment actions such as DHF related knowledge contest, DHF control innovation contest, PSN and PJB. The government of Cimahi also published regulations related to DHF and its vector Control.
5. **Capacity building.** There was no explicit document explaining about an activity with capacity building on its purpose. But the officers and community were getting information about DHF regularly through serial meeting and community empowerment.

REFERENCES

1. WHO. Pencegahan dan Penanggulangan Penyakit Demam Berdarah Dengue. Translated from WHO Regional Publication SEARO No.29. Prevention Control of Dengue and Dengue Hemorrhagic Fever. Jakarta: Depkes RI. 2000
2. Fuadiyah, M.Ezza azmi. *Analisis Biaya Manfaat Pelaksanaan Fogging di Kota Cimahi Tahun 2010.* Laporan Penelitian Riset Binaan Kesehatan. Balitbang Kemenkes. 2010.
3. Global strategic framework for Integrated Vector Management. Geneva: World Health Organization; 2004
4. Somantri, Gumilar R. Memahami metode kualitatif. Makara, Sosial Humaniora, Vol. 9, No. 2, Desember 2005: 57-65.
5. Basrowi, Suwandi. 2008. Memahami penelitian kualitatif. Jakarta: Rineka Cipta
6. Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan Depertemen Kesehatan RI. Pencegahan dan Pemberantasan Demam Berdarah Dengue Di Indonesia. 2005
7. Peraturan Menteri Kesehatan Nomor 374/Menkes/Per/III/2010 tentang Pengendalian Vektor.
8. Rozendaal JA. Vector control: methods for use by individuals and communities. WHO. 1997
9. Emmanuel Chanda, Fred Masaninga, Michael coleman, Chadwick sikaala, Cecilia katebe, Michael macdonald, et al. Integrated vector management: The Zambian experience. Malaria Journal 2008, 7:164
10. Beier JC, Keating J, Githure John I., Macdonald Michael B., Impoinvil Daniel E., Novak Robert J., et al. Integrated vector management for malaria control, review. Malaria Journal 2008, **7**(Suppl 1):S4

THE EFFECTIVENESS OF MOMORDICA LEAVES (*Momordica charantia L*) EXTRACT AS BIOLARVICIDE TO VECTOR OF DENGUE HAEMORRHAGIC FEVER

Lisda Hayatie[1], Istiana[1], Rahmiati[2], Maulana Sholihin[1]
[1]Department of Parasitology Medical Faculty of Lambung Mangkurat University
[2]Department of Microbiology Medical Faculty of Lambung Mangkurat University
Correspondence: lisdafkunlam@yahoo.co.id

ABSTRACT

Background: Momordica is a creeping plant which is popular known as traditional medicine to cure cough, intestinal worms, malaria and diabetes mellitus. Momordica plant also has a function as bioinsecticide to control *Aedes aegypti l*arvae. This research aimed to determine the effectiveness of Momordica leaves extract in killing *Aedes aegypti* larvae.
Method: This was an experimental study with post test only with control group design. The concentration of leaf extract was 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% along with positive control (temephos) and negative control (ethanol) with 3 times replicate. Momordica leaves were extracted maceratively.
Results: The result showed that momordica leaves extract has a significant influence to the mortality rate of larvae (p=0.000). Duncan experiment (Duncan Multiple Range Test) showed that all treatment were apparently different. The result of Probit Analysis showed that concentration of 23.9% of momordica leaves extract could cause death of 50% *Aedes aegypti* larvae (LC_{50}).
Conclusion: Extract of momordica leaves extract is effective as biolarvicides to *Aedes aegypti* larvae.
Keywords: momordica leaves extract, biolarvicide, *Aedes aegypti*

BACKGROUND

Dengue Hemorrhagic Fever is one of the public health problems [1]. Based on data from the Ministry of Health, dengue cases in Indonesia throughout 2012 as many as 90.245 [2]. in South Kalimantan, throughout 2012 of years, dengue case reaching 1216 cases. Various efforts to reduce morbidity and mortality has been done. One effort is to break the chain of transmission by inhibiting the proliferation of the *Aedes aegypti* mosquito which is the vector potential [3].

People use different ways to kill adult mosquitoes and larvae. One of them is larvicide, which has temephos as active ingredient, to kill larvae in water reservoirs. But there is an ongoing concern that temephos may lead to resistance. Therefore, it is necessary to find environmentally friendly alternatives bioinsectiside which is expected to have effects such as chemical larvicides [4].

Momordica are the plant is often used as one bioinsectiside. This bioinsectiside properties thought to be caused by the content momordicin compounds, alkaloids, flavonoids, triterpenoids and saponins which acts as a stomach poison for mosquitoes [5]. This study aimed to determine the effect of leaf extract of Momordica in a deadly

mosquito *Aedes aegypti* larvae and determine the optimal concentrations that kill 50% of larvae. This study is expected to yield scientific information about alternative larvicides from natural ingredients that can help suppress populations of *Aedes aegypti* larvae

METHOD

This was an experimental study with Posttest-Only Control Group Design. There were 11 treatments with 3 repetitions. The subjects were larvae of *Aedes aegypti* instar IV, because they have better defense system comparing to I, II and III [6]. The independent variable was concentration of leaf extract of momordica, which was devided into 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% respectively. We used as many as 25 larvae for each treatment. The dependent variable was the *Aedes aegypti* larval mortality after administration of momordica leaf extract for 24 hours. Room temperature was adjusted to 27^{o}C, which is a normal temperature for larval development [7]. The procedure consisted of extracting momordica leaf by maceration technique, and then testing these extracts larvicidal effect. The workings in this study in accordance with the method set by the WHO for experiments in the laboratory. Data were analyzed by ANOVA test, followed by Duncan and final Test using probit analysis through SPSS program.

RESULT

Figure 1 showed percentage of larval mortality after administration of the extract of momordica leaves 3 times repetition.

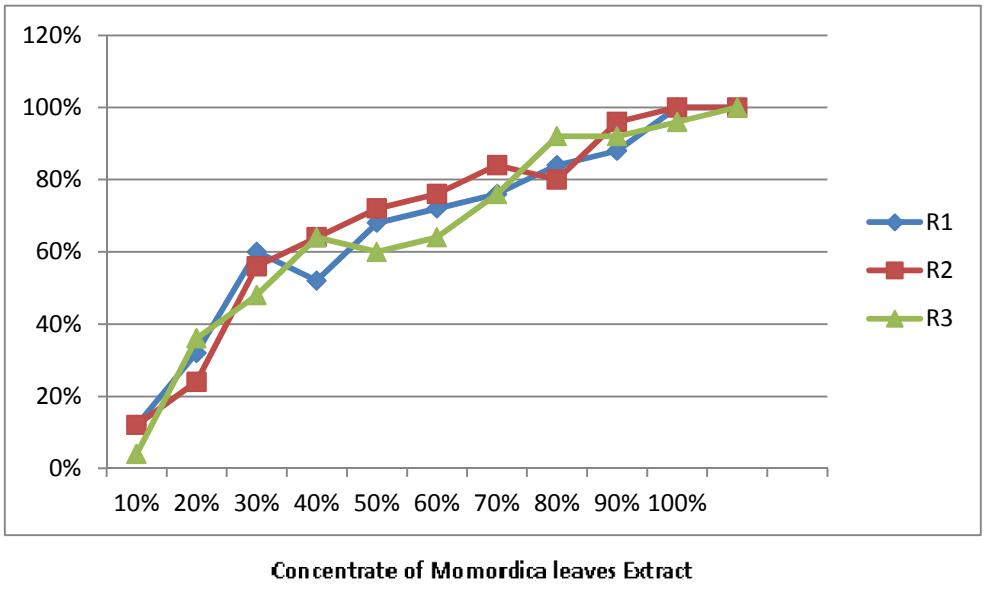


Figure 1. The percentage of larvae mortality after administration Momordica Leaf Extract with 3 times repetition in 24 hours.

The average percentage of larval mortality after administration of the leaf extract of momordica for 24 hours shown in Figure 2. As seen in Figure 2, mortality in control group (marked as group 1) was 9.3%.

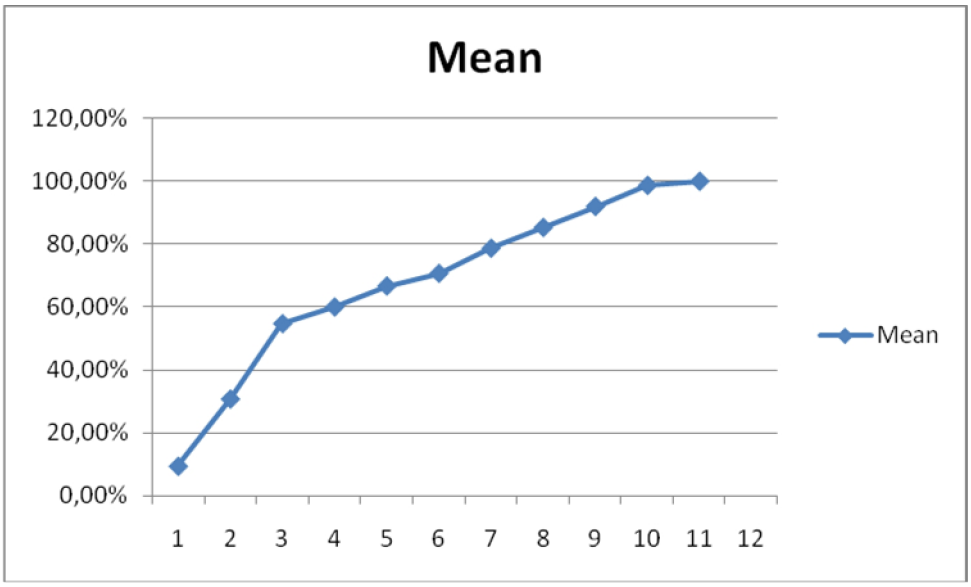


Figure 2. The average percentage of larvae mortality after administration Momordica Leaf Extract after 24 hours.

Since the mortality in control group was in range 5-20 %, the mortality of larvae must be corrected by Abbot's formula. Correction results shown in Figure 3.

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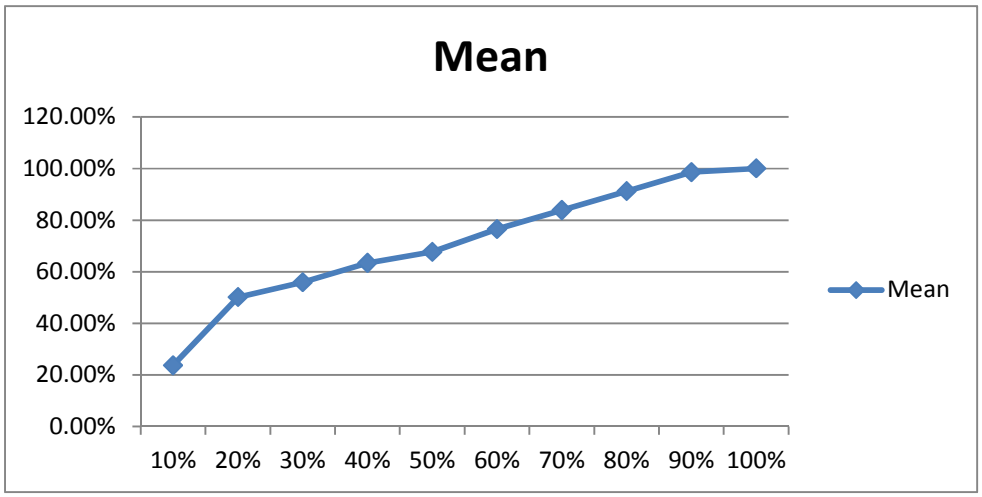

Figure 3. The average percentage of dead larvae after corrected with Abbot formula

Figure 3 shows the average percentage of mortality after correction with Abbott. Once corrected, the figure showed average percentage of dead larvae become smaller. To determine its effectiveness, the analysis of data to assess the homogeneity and the generated p=0.066, which means that the data are normally distributed. Data were futher analyzed with ANOVA to analyze different average mortality of larvae at various concentrations and the result $p<0.0001$, which means there is a significant amount of larval mortality between the treatment groups. To see the difference in the average mortality of larvae at various treatments can be determined by Duncan test (Duncan's Multiple Range Test). The results showed that all treatments were significantly different.

DISCUSSION

Figure 1 shows that the percentage of larval mortality after administration of the leaf extract of momordica for each concentration and repetition is quite varied, although the higher the concentration, the percentage of mortality is also higher. Similar condition also revealed from Figure 2. The results are consistent with Silfiyanti and Kristianto [8] which also showed that the higher the concentration of the extract given, the more dead larvae of *Aedes aegypti*. It is proved that momordica containing active compounds like momordicin, alkaloids, flavonoids, triterpenoids and saponins [5] can be used as larvicides. The working mechanism of momordica as larvicides is a stomach poison. If these compounds into the body of larvae, the appliance will be impaired digestion and inhibits taste receptors in the mouth larvae. This causes the larvae failed to get a stimulus and is unable to recognize the larval food and consequently died of starvation [8]. Active compound contained in the leaves of momordica also serves to inhibit the growth of larvae, which is the hormone inhibits the brain, edikson hormone and growth hormone (juvenile hormone). Due to disruption of the hormone, the larvae will not develop into adult mosquitoes and reduced coordination of movement, resulting in the death of the larvae [8].

The results of this study showed that the concentration of 10% leaf momordica extract has been able to kill larvae by 30.7%, and the average is significantly different from the average mortality at a concentration of 20% and so on up to the highest concentration of 100%. These results suggest that momordica leaf extract on the lowest dose capable of killing larvae already. The higher concentration of leaf extract, larval mortality was also greater.. Based on the criteria of efficacy is said that an extract effective if it is able to kill> 80% of the larvae within 24 hours after exposure [8], it can be said pare leaf extract effectively kills larvae at a concentration of 70% for this extract resulted in> 80% mortality of larvae.

To determine lethal concentration (LC) 50% using probit analysis. Probit analysis of the test results showed that the leaf extract of momordica at the lowest concentration of 18.2% can result in death instar IV larvae by 50%. While the upper limit of LC50 is 28.6%. So in this study can be determined LC 50 which effectively kill 50% of larvae was 23.9%. From the results of this research known that concentrations can kill instar IV larvae of *Aedes aegypti* was quite low at only 23.9%. This is in contrast with Silfiyanti and Kristanto [8] that showed that the concentration of 400 ppm, leaf extracts of momordica is able to kill 55% of larvae. Although different, these

results suggest that the leaf extract of momordica is proven laboratory has larvicidal effects.

CONCLUSION

Momordica leaf extract has the ability to turn off the fourth instar larvae of *Aedes aegypti*. The higher the concentration, the greater the percentage of his death. Concentration of Momordica leaf extract which killing 50% instar IV larvae of *Aedes aegypti* was 23.9% after 24 hours of exposure. However, further study needs to be done to determine the toxicity momordica larvicidal effect so hopefully this can be used as biolarvasida in society without any side effects and environmentally friendly

REFERENCES

1. Fathi, Soedjajadi K, Chatarina UW. 2005. Peran faktor lingkungan dan perilaku terhadap penularan demam berdarah dengue di Kota Mataram. Jurnal Kesehatan Lingkungan. 2:1-10.
2. Dinas Kesehatan Provinsi Kalimantan Selatan. 2013. Profil Kesehatan Daerah Provinsi Kalimantan Selatan tahun 2012. Hal :46-47
3. Gafur A, Mahrina, Hardiansyah. 2006. Kerentanan larva Aedes aegypti dari Banjarmasin Utara. *Bioscientiae* 3(2) : 73-82
4. Depkes RI. 2003. Prevention and Control of Dengue Haemorrhagic Fever. Jakarta : Departemen Kesehatan RI
5. Thorne Research Inc. 2007. Momordica Charantia (Bitter Melon). Alternative Medicine review.12 : 360—363
6. Banjo AD, Lawal OA, Aina SA. 2006. Insect Associated With Some medicinal Plants in South Western Norwegia. International Digital Organization for Scietific Information. 1(1) : 40-53
7. Susanna D, Rahman A, Pawenang ET. 2003. Potensi daun pandan wangi untuk membunuh larva nyamuk Aedes aegypti. Fakultas Kesehatan Masyarakat Universitas Indonesia. Jurnal Ekologi Kesehatan 2(2) : 228-231.
8. Silfiyanti E, Kristanto H. 2006. pengaruh pemberian ekstrak daun pare (Momordica charantia L) dalam menghambat pertumbuhan larva nyamuk Aedes aegypti. Malang : Universitas Brawijaya.

SURVEILLANCE OF *Plasmodium vivax* MALARIA TREATMENT AND PATIENTS COMPLIANCE IN TAKING MEDICATION TOWARD MALARIA ELIMINATION PROGRAM

M. Arie Wuryanto, M. Sakundarno Adi
[1]Faculty of Public Health, Diponegoro University, Semarang, Indonesia
Correspondence: arie_wur@yahoo.com

ABSTRACT

Background: Malaria remains health problem in Banjarnegara. According to the local District Health Office, there were 843 cases of malaria in 2011. Some of the health centers had experienced outbreak, dominated by *Plasmodium vivax* malaria. This indicated an occurrence of relapse**.** One of factors influencing relapse is non-compliance patients in taking medication. The compliance can be seen from surveillance of treatment. The aim of this study was to describe the surveillance of *P. vivax* malaria treatment and patients' compliance in taking medication.
Method: This was an observational study with cross-sectional design. Sample consisted of 126 patients for surveillance of malaria vivax treatment and 28 cases of malaria vivax for compliance in taking medication. Both were from Health Center of Wanadadi 1 and Banjarmangu 1. Data was collected by interviewing health officers (used surveillance form) and malaria vivax patients (used structured questionnaire).
Results: The implementation of surveillance of malaria treatment at Wanadadi 1 Health Center revealed that 60% cases were recorded. Of this, only 38.3% was recorded in complete information. At Banjarmangu 1 Health Office, no single patient was recorded nor observed in the patients' card for surveillance of treatment. Compliance in taking medication was only observed in 32.1% *Plasmodium vivax*, and 53.6% of patients had experience of getting previous vivax malaria.
Conclusion: Compliance of patients in taking medication and surveillance of vivax malaria treatment were not appropriately implemented in Banjarnegara. It is recommended to do the training for surveillance staff and perform assistance to the patients in taking medication.
Keywords: compliance, *Plasmodium vivax* malaria, surveillance of treatment

BACKGROUND

Ministry of Health of Indonesia through the Directorate General P2PL in 2012 informed that the cases of malaria in Indonesia remains need serious attention. API (Annual Parasitical Index) in 2011 was 1.7 / 1,000 population , the number of cases of malaria in 2011 was 256 592 people by the number of deaths was 288 people [3]. While the Information of malaria cases in Central Java province in 2011 the range of 19.454 cases [5].

Banjarnegara district as one of the endemic areas in Central Java province had experienced the impact of the success of the RBM , where in 2000 some high endemic districts, reach 100 more cases each month, then in 2005 the region has no cases [6].

However after 2007 until now, the incidence of new cases began to show improvement [2]. Banjarnegara district is malaria-endemic area, including vivax malaria. Vivax malaria cases from 2012 until February 2013 a total of 295 cases, with a high number of cases occurred in the area of Wanadadi 1 health center were 70 cases, and the Banjarmangu 1 health center of 115 cases. Wanadadi 1 Health centers have the largest percentage of vivax malaria cases (91 %), while at the Health Center of Banjarnegara 1 is 43 % positive cases of vivax malaria [7].

Un-usual events (Outbreak) vivax malaria was occured at Wanadadi Health Center with 45 cases, all cases was vivax malaria, whereas by 2012 through June 2013 there were 76 cases, and the proportion of cases with 90.8 % vivax malaria [7]. Outbreaks in which the malaria vivax was dominant, perhaps caused by relapse of malaria vivax [4]. The nature of relapse vivax malaria can experience in a long time when treatment was not appropriately done [8,9]. Some causes of relapse cases among others due to poor compliance patients in taking the medication and treatment surveillance is not running well [10,11].

Based on the above explanation, it can be formulated several research questions as follows: How about the implementation of surveillance malaria vivax treatment and compliance of vivax malaria in taking medication?

METHOD

This was an observational study with cross-sectional design. Sample consisted of 126 patients for surveillance of malaria vivax treatment and 28 cases of malaria vivax for compliance in taking medication. Both were from Health Center of Wanadadi 1 and Banjarmangu 1. Data was collected by interviewing health officers (used surveillance form) and malaria vivax patients (used structured questionnaire).

RESULT

Surveilans of treatment

In Wanadadi 1 Health Center january until June 2013 there were 77 cases of vivax malaria, whereas in Banjarmangu 1 health center there were 126 cases of vivax malaria. Based on Table 1, the number of patients with vivax malara observed to identify the surveillance of malaria treatment was 77 cases in the Wanadadi I Health Center and 126 cases in the Banjarmangu I Health Center. Of 77 cases in the Wanadadi I HC, only 60 cases (77.9 %) were written or recorded in patient card, and of this, only 23 (38.3 %) patients were recorded with complete recorded. While surveillance of malaria treatment at the health center Banjarmangu was worse. There was no record of treatment in patients cards at all. Monitoring and recording of treatment at the wanadadi I Health Center relatively better than Banjarmangu I Health Center.

Table 1. Number of cases and patient card (surveilans form)

Health Center	Wanadadi 1	Banjarmangu 1
Number of malaria vivax	77	126
Number of patient card	60	0
Percentage of patien card	77.9	0
Number of complete information	23	0
Percentage of complete information	38.3	0

Results of in-depth interviews obtained some interesting information related to the above phenomenon. Although some village malaria officers (*juru malaria desa*/JMD) did monitor the treatment, they did not record all observation results on the patient card. However, some JMD have taken a note in the logbook. Results of follow up (re-examination) of blood slides also been done, although not on time as scheduled.

Some of problem the recording and monitoring treatment of vivax malaria patients are: (a). Monitoring and implementation of the follow-up (re-examination) of blood slides had done, but sometimes in the home visits, the patient was not at home. This would disrupt the schedule of the next visit, and finally often forgotten because of unsystematically records in JMD's logbook. It did not distinguish new patient examination or follow-up results. So, it is too difficult for JMDs to do the evaluation themself, whereas the monitoring of treatment results was under their responsibility. Moreover, number of JMD /in Banjarmangu I Health Center was lacking, so the re-monitoring of vivax malaria treatment was rarely done. This is because most JMD has become a goverment staf at Health Center, and placed in another section; (b). Recording of patient treatment monitoring was not carried out on the card in Banjarmangu Health Center I, it is associated with the enforcement of discipline that was applied by the head of the health center; (c). Monitoring and evaluation of malaria programs conducted by the health center or district health office have not emphasized on the treatment surveillance (monitoring records of treatment and re-survey of blood slides) to ensure vivax malaria patients take medication completely and totally cured.

Patient Card of vivax malaria is one of the instruments used in the surveillance of malaria treatment. The instrument can be used to anticipate the spread of malaria transmission caused by incomplete treatment. Therefore the card is designed to monitor/supervise the implementation of the patient in treatment. The patient card of vivax malaria contain information as follow: (a). Patient identity consisted of name, gender, age, occupation, and address; (b). History of malaria consisted of the date of blood smear collection, date of clinical sypmtom, date of blood clots inspection, and date of radical treatment; (c). Follow-up consisted of the date of follow-up, date of blood smear collection, preparation and examination of blood; (d). Types of *Plasmodium*.

Compliance in Taking Medication.

Information of compliace treatment based on interview and validation from recording treatment through patient Card information.

Table2.Distribution of Compliance

No	Compliance	n	%
1	Yes	19	67.9
2	No	9	32.1
Total		28	100.0

Based on table 2, there were 32.1% not-compliance patients. Based on this information of compliance, we will know there is potential relaps of malaria vivax in the next time periode. Some problem to ensure patient will be compliance in taking medication were: (a). Patient will be get feelings better after 3 or 4 day medication, and so wil be stop continuing of medication; (b). Patient getting side effect such as nausea, dizzy and unwell when taking medication; (c). Perceive of patient that taking medication for up to 14 days is considered very boring.

Table3. Distibution of level knowledge

No	Level of knowledge	n	%
1	Good	22	78.6
2	Not Good	6	21.4
	Total	28	100.0

Table 3 showed most patient (78,6%) have a good knowledge of medication, but 21,4% patient getting not good in knowledge of medication. While table 4 showed there ware 53% patient have experience of vivax malaria in the previous.

Tabel 4.Previous Experience of Vivax Malaria

No	Previous	n	%
1	Yes	15	53.6
2	No	13	46.4
	Total	28	100.0

DISCUSSION

Based on information of surveillance medication through observation of patient card records, then we can see that the surveillance of treatment has not been going well in a both health center, especially in Banjarmangu I Health center. Only 77.9 % of patients were recorded in the patient card, and only 38.3 % was recorded in full reported by the results of follow-up treatment. On the other side, no patient card was used at the Banjarmangu I health center.

Some benefits from the use of the card were the ability to determine the characteristics of patients with malariathe greater proportion of *Plasmodium* species in that area. Thus, the card can monitor wheter health officers carry out the follow up or not. Therefore the impact of usage of patient card can describe by the number of

patients with complete follow-up and number of patients with incomplete follow-up in an area.

Complete treatment will have good monitoring if the card was filled completely. Compliance of treatment can guarantee complete cure to vivax malaria, and eliminate sources of transmission. Complete treatment can be monitored well if the card filled completely in accordance with the terms of use. Monitoring of malaria patients who have received radical treatment can assess the effects of drug, so the drug resistance of medication can be avoided. Monitoring through patient card malaria by taking repeated blood preparation/follow-up of patients was able to know if the medication given an expected results (patients' recovery or elimination of parasites in the re-examination of blood). If the medication revealed not as expected result then the drug will need to be analyzed to ensure whether it was already resistant.

The low implementation of surveilans treatment will interfere the monitoring of patient compliance in taking medication to ensure healing of patient. Follow-up examination of blood slides that should be done on day 3, 7, 14, 28 and month 3 will be able to ascertain whether the patient is completely cured or not. Another benefit of the follow-up is an opportunity officer for more frequent contact with the patient, so that it will be easier to giving motivation, which will improve patient compliance in taking medication.

Despite 67.9 % of patients who werre adherent (compliance), there remained 32.1 % of patients who were not adherent. The conditions triggered vivax malaria transmission in the region by relapse of vivax malaria patients. Therefore, this is one of the potential barriers to achieving the target elimininasi malaria in the future.

CONCLUSION

Compliance of patients in taking medication and surveillance of vivax malaria treatment were not appropriately implemented in Banjarnegara. It is recommended to do the training for surveillance staff and perform assistance to the patients in taking medication.

REFERENCE

1. Dinkes Kab.Banjarnegara, "Profil Kesehatan Kabupaten Banjarnegara, tahun 2011.
2. Dinkes Kab.Banjarnegara. Laporan Bulanan Pengobatan dan Penemuan Penderita. Banjarnegara: Dinas Kesehatan Kabupaten Banjarnegara; 2011.
3. Departemen Kesehatan RI, Malaria : "Modul Parasitologi Malaria", Direktorat Pemberantasan Penyakit Bersumber Binatang, Jakarta. 2012.
4. Widoyono. Penyakit Tropis. "Epidemiologi, Penularan, Pencegahan, dan Pemberantasannya". Jakarta: Penerbit Erlangga; 2008.
5. Dinkes Prov.Jateng, "Profil Kesehatan Provinsi Jawa Tengah tahun 2012.
6. Wuryanto A., "Beberapa Faktor Risiko Kepatuhan Penderita Malaria Vivax Dalam Minum Obat", Thesis, Magister Epidemiologi UNDIP, Semarang, 2005.
7. Dinkes Kab.Banjarnegara. "Laporan Bulanan Pengobatan dan Penemuan Penderita. Banjarnegara Tahun 2012."
8. Harijanto, P.N., 2000, Malaria : "Epidemiologi, Patogenesis, Manifestasi Klinis, dan Penanganan", Cetakan I : EGC, Jakarta. h.37-62.

9. Prof. dr. Tjandra Yoga Aditama, “Gebrak Malaria Dalam Rangka Peringatan Hari Malaria Sedunia Tahun 2012.” http://www.pppl.depkes.go.id .
10. WHO, “ Roll Back Malaria : What Are The Prospect ”. Buletin of the WHO, 18 (12) : 1377.2000.
11. Chin, James, “Control of Communicable Diseases Manual”, 17th Edition, American Public Health Association 800 I Street, NW, Washington. DC 20001-3710. p. 310-322. 2000.

ANALYSIS ON KNOWLEDGE AND ATTITUDE OF EMPLOYEES' PRACTICESIN APPLYING BBS (BEHAVIOR-BASED SAFETY)PROGRAM AT PESTICIDE FORMULATION COMPANY

Taufiq Ade Elita, Yuliani Setyaningsih, Bina Kurniawan
Occupational Safety and Health, Faculty of Public Health, Diponegoro University
Email : joeliani_kesja_undip@yahoo.com

ABSTRACT

Background: Many efforts have been done to create a healthy workplace and accident prevention, including the introduction of behavior-based safety program (BBS), by changing unsafe into workers' safe behavior. Pesticides Formulation Industry in Semarang has been applying BBS since 2005, but the implementation was not optimal. There were some problems in communication and only permanent employees implemented BBS program. The aim of this study was to analyze knowledge and attitudes of the employees in implementing BBS program (Behavior-Based Safety) in pesticides formulation industry in Semarang.
Method: This study used qualitative method by in-depth interviews, observation and document review. Subjects were 6 employees in Pesticides Formulation Industry in Semarang. Triangulation informants were the supervisor and SHE officer.
Result: The results showed that the problems in the implementation of BBS program in Pesticides Formulation Industry in Semarang were: the workers only fulfilled their tasks to get award; BBS checklist items were unspecialized for each profession and unit production; lack of managers attention for non permanent employees. The results also indicated that subjects' knowledge were not correspond to their attitude and practices in BBS program.
Keywords: Knowledge, Attitude, Practice, Behavior Based Safety (BBS)

BACKGROUND

Occupational Safety and Health (OSH) is an effort to protect workers, the company, the environment and the surrounding community from exposure to hazards in the workplace. In the era of globalization requires the implementation of Occupational Safety and Health (OSH) in every workplace including in industry, it is necessary to develop and improve the OSH in order to minimize the risk of accidents and illness arising from work, as well as increase productivity and efficiency [1].

The accidental of work can occur because of two main factors : unsafe behavior and unsafe condition. According to data of "Biro Pelatihan Tenaga Kerja", the causes of work accidental are unsafe condition such as careless behavior, do not comply with regulations, incompliant of work standard procedure, not wearing personal protective equipment, and weak physical condition. Percentage of causes of accident are 3% due to reasons that can not be avoided (such as disaster), 24% due to environment or equipment that do not qualify, and 73% due to unsafe behavior. Effective way to prevent accidents is to avoid the occurrence of five unsafe behaviors mentioned above [2].

Various attempts have been made to create a healthy workplace and safe from accidents, include introduce a behavior-based safety program or BSS, which emphasizes the aspects of unsafe behavior [3]. The purpose of the Behavior-Based Safety is changing the behavior of employees from unsafe behavior into safe behavior [4]. The behavior-based safety techniques consist primarily of employee training on safe and risky behavior, observation, intervention and feedback [5,6].

BBS program requires the cooperation and participation of workers, since the program is aimed at changing the behavior of the workers themselves. Behavior of individual workers in the company are very different from each other in the manner and under certain conditions, to change the behavior of workers who initially had a habit takes a long time and is not easy in implementation.

Companies of pesticide formulation have been using the BBS since 2005. BBS program before being applied to the employee, in advance socialized with training on employee. However, in its application is not maximized, seen from the incidence of first aid and near misses during the year 2011 in the Company of pesticide formulations monthly is likely to increase. Up to 73.68% of the cases occur as a result of unsafe behavior of employees. Therefore, purpose of this study was to analyze the knowledge and attitudes with employee practices in implementing BBS (Behavior-Based Safety) in pesticide formulations company in Semarang.

METHOD

The research method used was a qualitative-oriented method with inductive logic (getting the idea by examining inductively from specific data to more general data) [7]. The purpose of qualitative research is to develop concepts that help further understanding the social and behavioral phenomena in the natural background [8].

Purposive sampling was used to select study subjects with criteria as follow: (a) Permanent employees in the production department which included the BBS program; (b) Had attended training or training on BBS; (c) Men or women; (d) Willing to provide information when interviewed. Number of study subjects were 6 employees in the production department, because of the six employees have obtained appropriate information and research purposes of data saturation occurs (saturation information).

The main data sources in qualitative research is the words and actions, the rest is additional data such as documents and other [9]. Data collection used interview, observation, documentation writing, and literature. Means of collecting data in the form of an interview guide to measure knowledge and attitudes, while the practice of using a checklist to measure.

Triangulation is a technique that utilizes data validity checking something else out that data for checking purposes or as a comparison to the data. Triangulation with source can be achieved by comparing the employees covered by the BBS program and supervise officers of SHE.

RESULT

BBS program (Behavior-Based Safety) in pesticide formulations applied to the Company since 2005 with reference to the Occupational Health and Safety Resource Manual that is used by the industry. Coverage of the program BBS (Behavior-Based Safety) in company of pesticide formulationare permanent employees in all areas of the company, the contract length that employee's annual contract period, while contract employees in this short-term contracts are not included in the monthly contract implementing BBS program. Any rewards for employees who are able to assess the BBS on schedule, which is 2 times during the months of the year. BBS program overall shape in the Chemical Industry includes assessment of BBS (Behavior-Based Safety) employees in the form of employee behavior observation or observation made by others, after the observation of some employees collected later in the recap into one by grouping any unsafe behavior the employee, how the response or feed back employee was observed, and the recommendation of the observer.

Results of in-depth interviews were conducted to study subjects related to knowledge of the subject of the application BBS program in pesticide formulations companyknown that, all study subjects had knowledge of the BBS program. This is evident from the study subjects were able to answer and explain the definition, measures, and how the implementation of the BBS program.

The attitude shown through the responses of research subjects, assessment and opinion on the implementation of the BBS program is also supportive, accepting and implementing the program with the assumption that the BBS program aimed at employees own safety. Despite the BBS program will increase the workload, eventually research subjects willing to accept and implement the BBS program for reasons of an assignment or order of management. In addition, because the rewards make employees perform BBS program in order to find the reward.

Result of assessment of the 6 subjects of research on the practice of application of the BBS program, only 2 subjects that is fully safe behavior as a form of implementing BBS program. Most of the research subjects is not fully safe behavior is a form of practice level who have not been able to do something with the settings automatically, or something is not a habit.

Most subjects showed no correspondence between knowledge with practice in applying the BBS program. From interviews regarding knowledge, study subjects were able to answer and explain how the implementation of the BBS program. While in practice, most of the study subjects have not fully implemented the BBS program outcomes assessment in terms of safe behavior observation. The results also showed that attitude of research subjects included in the definition does not fit or do not depend on each other to practice. Though being contributed, accept and implement the BBS program, but in practice does not fully implement the program. The research subject is only responsible for the reason of the assignment or order management. When a command is part of the task, the research subject was going to carry out. In addition, the rewards for those who filled the form BBS made the subject simply fill out the form BBS. Ultimately the responsibility to implement and properly familiarize BBS program has not materialized.

DISCUSSION

The sense Behavior based safety (BBS) is the use of behavioral psychology to promote savety [10]. BBS programs aim at the company of pesticide formulations in line with the understanding Behavior -Based Safety by Geller, an application of the behavioral sciences that deal with safety issues at work. Behavior based safety focuses on what others are doing, and then analyze why they are doing it and finding appropriate interventions to improve the ability of the person. BBS is usually used to change the behavior of workers insecure behavior become safe behavior to prevent accidents. So, with the increase in safe behavior also will be achieved the success [4]. This pesticide formulations company employs more contract workers than permanent employees, but the implementation of the program BBS (Behavior-Based Safety) only applied to permanent employees in all areas of the company. Employees who conduct assessment BBS (Behavior-Based Safety) is appointed and training or training in BBS.

Assessment the BBS (Behavior-Based Safety) schedule of employee has been determined by the section Safety, Health and Environment at the company, which is 2 times every month. Observers will get a different area or perform rolling/change place every month in assessing BBS. Safety requires a collaborative process where everyone in the company to participate to create a safer workplace, especially given that the company produces toxic substances that are harmful to human health. With the growing culture of engagement and participation, can be achieved zero injuries. Involvement in safety is ultimately benefit employees and the organization. Every worker has something meaningful to contribute, and people will contribute if the climate is right [13].

All research subjects had good knowledge about the BBS program. Indicated that the subjects were able to answer and explain the definition, measures, and how the implementation of the BBS program. This is consistent with the results of Latifatul Mufarokhah that indicates a link between safety knowledge with the implementation of accident prevention [11].

Attitude shown through the responses of research subjects, assessment and opinion on the implementation of the BBS program is also supportive, accepting and implementing the program with the assumption that the BBS program aimed at employees own safety. Although the BBS program will add to the burden of work, research subjects willing to accept and implement the BBS program for reasons of an assignment or order of management. Companies provide rewards to employees who perform BBS program. It is also one of the things that encourages employees want to implement this program. This result is in line with research Yusri and Johnny Situmorang stated that the attitude towards safety in industrial environments showed a good response, although there are some good responses but are less likely to be many who showed a good response to occupational safety [12].

Most subjects showed no correspondence between knowledge with practice in applying the BBS program. Based on the results of the interviews showed that the expression of knowledge study subjects were able to answer and explain how the implementation of the BBS program. But the safety assessment of the behavioral observation study subjects showed most of the study subjects have not been fully implemented BBS program. This result is in line with research showing that

Mahachandra and Manik, OSH knowledge did not correlate significantly with the implementation of OSH management [13].

Attitude research subjects included in the definition of nonconformity or not independent of one another with his behavior. When linked with the practice in implementing BBS, attitude is subject of a study contribute to support, accept and implement the BBS program, but in practice does not fully implement the program. The research subject is only responsible for the reason of the assignment or order management. In addition, the rewards for those who make the subject fill the BBS just fill out the form.

Knowledge levels of the subjects included in the study can answer and explain it. Research subjects can recall previously learned material, in this case is the BBS program. In addition, the research subjects were able to explain, cite examples in the BBS program and implement the company. Most of the new subjects are at levels not describe the next level of analysis, ie the ability to describe the BBS program material that has been studied according to the situation or the actual condition.

Attitude on the subject of new research levels receive, respond to and appreciate the application of the BBS program, not to be responsible for the implementation of a voluntary program and not make it as a basic requirement in safety work. Although the research subjects applying one form of form filling activity BBS, it was not because of the awareness of the urgency of the BBS program, but for some reasons such as: (a) a tendency toget reward; (b) willingness to participate in our study only by reason of the assignment or order management. In addition, the reward for a full charge, making the subject simply fill out the form BBS. There is no responsibility to familiarize implement the BBS program and practicing safe. DePasquale and Geller mentioned five variables significantly predicts meaningful employee involvement in the process of BBS: (1) The perception that BBS training is effective; (2) Belief in the ability of management; (3) Accountability or responsibility for the BBS through performance assessment; (4) Whether a person receives education on BBS; (5) The term of office with the organization [14]. So the factors that affect the implementation constraints BBS program at the company's pesticide formulations because of their responsibility for the BBS have not really in order to familiarize the BBS, but the chase and meet the award provided by the company.

According to the informant triangulation, lack of programs such as the items in the checklist BBS BBS is too much, the lack of attention from management to employees short contracts, and less management attention in the follow-up data collection results from the application BBS program.

CONCLUSION

Description of program implementation BBS (Behavior-Based Safety) in pesticide formulations company is BBS program implemented since 2005, the BBS program coverage permanent employees and contract employees with long-designated assessment schedule BBS 2 times per month and each month rolling assessment locations, as well as there is a reward voucher when employees make an assessment on schedule with full BBS.

There is no correspondence between knowledge and attitude to the practice of the majority of the research subjects in applying the BBS program. Because of the

knowledge that has not been able to describe the BBS program material that has been studied according to the actual situation or condition, reflects the attitude of the research subjects only to fulfill the duties and pursue awards provided by the company, so the practice does not fully implement the BBS program.

REFFERENCES

1. Budiono S. Bunga Rampai Hiperkes dan KK.2003.
2. IOSH. Materi Keselamatan dan Kesehatan Kerja Tenaga Kerja Asing - Bidang Elektronik. Diakses tanggal 02 Februari 2012 dari http://www.iosh.gov.tw/upload/netbook/foreign/960718-204.pdf.
3. Sakdiah MH, Gopal RK. The Effectiveness of Behavioural Based Safety Towards The Safety Performance In The Oil and Gas Industry In Malaysia. Journal of Community Health 2010. 2010;16(2):36 - 46. Diakses tanggal 02 Februari 2012 dari http://www.communityhealthjournal.org/pdf/Vol16(2)-Halimatus.pdf.
4. Smith TA. What's Wrong With Behavior-Based Safety? 1999:1-8. http://www.mocalinc.com/sitebuildercontent/sitebuilderfiles/whatswrongwithbehaviorbasedsafety.pdf. Accessed 09 March 2012.
5. Stranks J. Human Factors and Behavioural Safety. 1 ed: Butterworth-Heinemann, Elsevier Ltd; 2007.
6. Geller ES. The Psychology of Safety Handbook. 2nd ed: CRC Press: Lewis Publishers; 2001.
7. Poerwandari EK. Pendekatan kualitatif dalam penelitian psikologi. Jakarta: Lembaga Pengembangan Sarana Pengukuran dan Pendidikan Psikologi, Fakultas Psikologi UI; 1998.
8. Kustanto H. Metode Penelitian Kualitatif Dalam Riset Kesehatan. Yogyakarta: Program Studi Ilmu Kesehatan Masyarakat, Pasca Sarjana UGM.
9. Moleong LJ. Metode Penelitian Kualitatif. Bandung: PT Remaja Rosdakarya; 2007.
10. Riley R. How Behavior Based Safety Programs Work. ABC, Construction Education Conference. 2010. Diakses tanggal 21 Februari 2012 dari http://www.abc.org/files/Events/Construction_Education_Conference/How%20Behavior-Based%20Safety%20Programs%20Work.pdf.
11. Mufarokhah L. Hubungan Pengetahuan Keselamatan Kerja dengan Pelaksanaan Pencegahan Kerja pada Karyawan bagian spinning di PT. Primatexco Indonesia Batang. 2006. Diakses tanggal 20 Juni 2012 dari http://www.scribd.com/doc/55412984/Hubungan-Pengetahuan-Keselamatan-Kerja-Dengan-Pelaksanaan-Pencegahan-Kecelakaan
12. Yusri H, Situmorang J. Sikap terhadap Keselamatan Dari Pekerja Radiasi Di Lingkungan Rumahsakit Dan Industri Indonesia. Buletin Keselamatan STATUTA. 2000;1(1):8-14.
13. Mahachandra Jm, Manik Aas. Korelasi antara pengetahuan, sikap, dan praktik keselamatan dan kesehatan kerja (K3) dengan penerapan manajemen K3 pada PT Kereta Api (Persero) daerah operasi VI Jogjakarta. 2008. Diakses tanggal 20 Juni 2012 dari http://etd.ugm.ac.id/index.php
14. DePasquale J, Geller ES. Critical Success Factors for Behavior-Based Safety: A Study of Twenty Industry-Wide Applications. Journal of Safety Research. 1999;30(4):237-249.

MORPHOTYPE VARIATION OF *Aedes aegypti* INDENGUE HEMORRHAFIC FEVER ENDEMIC AREA OF SURABAYA. INDONESIA

Subagyo Yotopranoto[1,2], Kusmartisnawati[1], Heny Arwati[1], Kriscahyo Mulyatno[2], Rosmanida[3]

[1]Department of Parasitology, Faculty of Medicine, Airlangga University, Surabaya
[2]Entomology Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya
[3]Department of Biology, Faculty of Science and Technology, Airlangga University
Correspondence: arwatiheny@yahoo.com

ABSTRACT

Background: Dengue haemorrhagic fever (DHF), a mosquito-borne disease, is a major cause of morbidity and mortality worldwide, especially in tropical and subtropical regions. *Aedes aegypti* mosquito is a vector of this disease. Morphologically, there are white scales spread at the base of tergal's ribbon to distinguish morphotype of *Ae aegypti*. Little is known about morphotype of this mosquito. Therefore, observation of white scales distribution on adult mosquitoes has been done to find out the morphotype variation of *Ae. agypti* in DHF endemic area of Surabaya.
Method: Larval stage samples were collected from their breeding sites throughout 50 houses within 11 Rukun Warga of Nginden village. The collected larval samples were then reared to obtain adult stage aged one day in Entomology Laboratory in the Institute of Tropical Disease, Airlangga University, Surabaya. By observing the white scales pattern on the tergal of each abdominal segment on adult mosquitoes, seven morphotypes could be identified as morphotype 1 up to morphotype 7.
Results: The mosquitoes with morphotype 1 were identified as the greatest in number (67.2%), followed by morphotype 7-bearing mosquitoes (9.3%). Morphotype 2, 3, 4, 5 and 6 were found to be less than morphotype 7. Observation on distribution of each morphotype during the course of study showed the similarity of distribution among the three months observation. The most preferable breeding sites of *Ae. aegypti* found in the study area were water container in bath room (*bak mandi*) located either in indoor or outdoor. The fluctuation of *Ae. aegypti* larval population showed that the highest larval density population occurred in January (82.4 larvae/house), and gradually decreased in March (63.5 larvae/house), while the lowest occurred in May (21.1 larvae/house).
Conclusion: Seven morphotypes variation of *Ae. aegypti* was found. Investigation of morphotype variation of *Ae. aegypti* should be continued in dry season to observe the changing of variation and to find out which most potential morphotype transmitting dengue virus.
Keywords:*Aedes aegypti*, morphotype variation, larval density, season.

BACKGROUND

Dengue haemorrhagic fever (DHF), a mosquito-borne disease, is a major cause of morbidity and mortality worldwide, especially in tropical and subtropical regions. Dengue haemorrhagic fever is caused by dengue virus (Family Flaviridae, genus *Flavivirus*). This virus has four antigenic serotypes: Dengue serotypes 1, 2, 3 and 4

which are also called Den 1, Den 2, Den 3 and Den 4. The four serotypes have been found in Surabaya, but the dominant serotype was Den 2 [1,2].

Nearly 2.5 billion people in the world are threatened by this disease, 50-100 million people are at risk and possibly 21 thousand patients are died per year [3]. The presence of DHF in Indonesia was first reported occurred in Surabaya and Jakarta during 1968, where 24 patients died among 58 DHF-infected children (case fatality rate was 41%) [4]. Then, the disease spread to several cities in East Java Province due to less attention of the people to the environment (Head of Department of Health Surabaya, 2009). Nginden village of Sukolilo District, Surabaya city is a DHF endemic area. The first case of DHF was reported in 2004 and continuously increased until 2008.

Dengue virus was sucked by *Ae.aegypti* from DHF patient with viremia during blood meal [1]. The virus becomes infective in the mosquito's salivary gland and ready to transmit Dengue virus to the susceptible host through mosquito bite. Based on the color of the body, there are two kinds of *Ae.aegypti.* The bright color is active within indoor areas, another one is the dark color that active in outdoor areas and widely distributed in the bushes [5-7]. In general, the body of *Ae.aegypti* is black with white spots and lines. The lyre-shape mark on the dorsum of thorax is a specific feature of this mosquito. Lyre consists of one white curve line on each lateral and one white straight line in the median [8]

The abdomen of *Ae.aegypti* consists of 8 segments and on the dorsal part of each segment there is a white ribbon. White scales spread at the base of the ribbon. The distribution of white scales on tergal's ribbons is to distinguish the morphotype of *Ae. aegypti.* According to Mc Clelland [6] there are 8 morphotype of white scales: type 0 (no white scales), type 1 (white scales only on tergal of segment I), type 2 (white scales on tergal of segment I to II), type 3 (white scales on tergal of segment I to III), type 4 (white scales on tergal of segment I to IV), type 5 (white scales on tergal of segment I to V), type 6 (white scales on tergal of segment I to VI), and type 7 (white scales on tergal of segment I to VII). Distibution of the morphotype was shown on Figure 1.

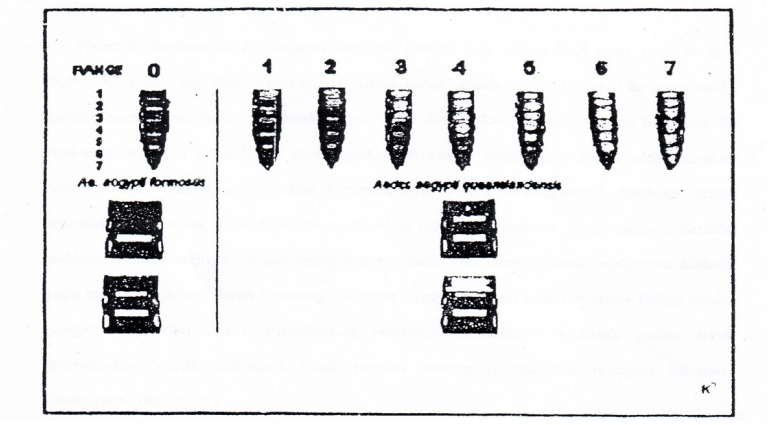

Figure 1. Pattern of scale variation on the tergal of abdomen segment of *Ae. aegypti* (Mc Clelland, 1974)

The objective of this study is to find out the population condition of *Ae.aegypti*, the morphotype variation and its distribution, and larval density index collected at Nginden village of Sukolilo District, Surabaya City during rainy season.

METHOD

Population

The populations of sample in this study are breeding sites of *Ae.aegypti* indoor and outdoor within residential houses in the study area, including larvae of this mosquito species inside breeding sites.

Sample and sample size

The sample is larva of *Ae.aegypti* which found in the breeding sites indoor and outdoor in study area. Samples were collected from 50 houses within 11 RW in Nginden village of Sukolilo District, Surabaya. Randomly 4-5 houses were chosen from each RW. Sample size is number of random sample of larva found in the breeding sites of chosen houses.

Samples collection

Larvae were collected by water dipper and count the number of larva found in each breeding site during rainy season of January, March and May 2009. Larvae were then put into container containing water before transporting them to the Entomology Laboratory at Institute of Tropical Disease Airlangga University, Surabaya prior to rear them in order to obtain the adult mosquito and morphotype identification.

***Larval density index* (LDI)**

TheLDI was determined by counting the number of *Ae. aegypti* larva within the number of investigated houses as follows:

$$\text{LDI} = \frac{\text{Number of larva}}{\text{Number of investigated houses}}$$

Morphotype identification

Adult mosquitoes obtained after rearing the larva were then dispatched in the refrigerator (10°C) for 15 minutes prior to morhotype identification. Morphotype was identified based on Mc Clelland [6].

RESULT

Table 1. Number of *Ae. aegypti* larva and LDI during the course of study

Month	Number of larva	LDI
January	4120	82.4
March	3175	63.5
May	1056	21.1

The breeding places of *Ae.aegypti* identified within 50 houses in Nginden village of Sukolilo District in Surabaya City during rainy season of January, March and May 2009 were water container in bath room and toilet, large water jar, water container attach to the refrigerator, bucket, aquarium, well and container for taking *wudhu* (the

Muslim activity before praying). The number of collected larvae from those breeding sites and LDI were seen in table 1. The adult mosquitoes were obtained from rearing of larvae collected in the study area during rainy season of January, March and May 2009. The morphotype were then identified based on the distribution of white scales on the tergal's ribbon on the dorsal part of abdominal segments, followed by counting the number of mosquito bearing the morphotype. Distribution of each morphotype was presented in Table 2.

Table 2. Distribution of morphotype pattern of *Ae. aegypti* in Nginden village, Surabaya

Month	Number of mosquito (%)						
	M 1	M 2	M 3	M 4	M 5	M 6	M 7
January	2760 (67%)	165 (4%)	82 (2%)	268 (6.5%)	222 (5.4%)	196 (4.7%)	429 (10.4%)
March	2177 (65.4%)	143 (4.5%)	95 (3%)	222 (7%)	187 (5.9%)	162 (5.1%)	289 (9.1%)
May	731 (69.2%)	43 (4.1%)	21 (2%)	65 (6.2%)	59 (5.6%)	45 (4.3%)	92 (8.7%)

M: morphotype

The data showed that *Ae. aegypti* in study consisted of 7 morphotypes, those are morphotype 1, morphotype 2, morphotype 3, morphotype 4, morphotype 5, morphotype 6 and morphotype 7. Based on the number of morphotype-bearing mosquitoes the interesting pattern has been found that morphotype 1 was always showed the highest number either in January, March or May. The first position of morphotype 1 was then followed by morphotype 7. This pattern of morphotype distribution was always found in three months of collection time.

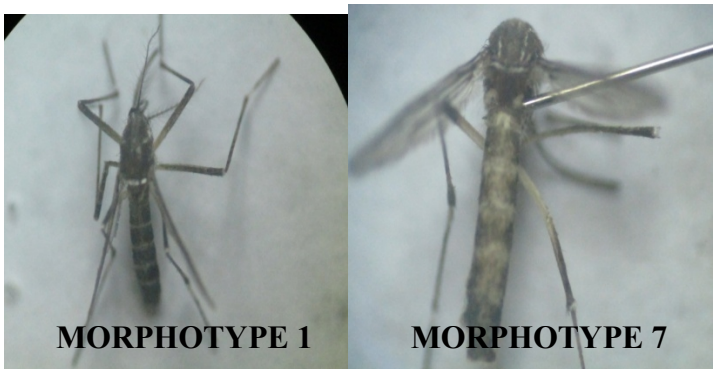

Figure 2. Pattern of morphotype of *Ae. aegypti* in Nginden village of Sukolilo District, Surabaya City during January, March and May 2009.

DISCUSSION

Dengue haemorrhagic fever (DHF) remains endemic in Surabaya. The presence of breeding sites are indeed supporting the life cycle of *Ae. aegypti.* Activity of the people

and physical environment influence the presence of breeding sites. Most of residential houses in study area were houses of middle and low society levels (70%) with high people density and the buildings of houses were so close to each other. Each house is occupied by 5-10 persons. Kind of breeding sites most found in study area were water container in the bath room (Indonesian language: *bak mandi*) (75%), followed by large water jar made from plastic, clay, or cement (16%) located either indoor or outdoor. People usually are keeping water in the bathroom for daily bathing, while for cooking they use water in the large jar. These two water containers are good breeding sites of *Ae. aegyti* but people do not realize that they keep dangerous mosquito in their houses.

Hasyim and Soekirno [9] found different condition of breeding sites in two different areas of Tanjung Priok in Jakarta. Large water jar was the most found breeding sites in the first area, but in the second area was water container in the bath room. People take water from an opened well and store in the large water jar for daily use. A survey in Petemon village of Surabaya a well can be a breeding site of *Ae. agypti* [10]. A report by Gionar et al [11]found that in Gondokusuman, a village in Yogyakarta city, during dry season 35% of well containing larva of *Ae. aegypti*, and increased up to 51% during rainy season. Several researches in Lao, Vietnam and Thailand showed that most of inhabitants use large water jar made from clay or cement for storing water. This kind of water container is the most found breeding site in those areas [7,12,13]. In Australia, roof gutter is important breeding site of *Ae. aegypti.* The more number of larvae of this mosquito are found in the roof gutter during rainy season rather than during dry season [14], However, in Surabaya roof gutter plays fewer roles as breeding site of *Ae. aegypti* as found that only 6.6% of larva found in this breeding site [15].

In the current study area, Nginden village of Surabaya city, the highest density of *Ae. aegypti* larva was found in January (Table 1) compared with that found in March and May. January is the peak of rainy season in Surabaya where population *Ae. aegypti* increase due to the high humidity, the warm air temperature, and the high rainfall resulting in water pool in the house yard that potential as mosquito breeding site. While the density of larva decreased gradually as shown by the LDI in January (82.4 larva/house), to March (63.5 larva/house) and May (21.1 larva/house) as well as the decrease of rainfall. The increased of people awareness to clean the water container in their house was also played role in the decrease of larva density in this area as they enlightened by the researchers to avoid the occurrence of mosquito's breeding sites.

Generally, *Ae. aegypti* consists of two groups of mosquito. The first group is *Ae. aegypti formosus*, that characterized by dark color and lack of white scales on the dorsal part of abdomen. This group prefers to live in the bushes in outdoor. The second group is *Ae. aegypti aegypti* or *Ae. aegypti* var *queens landensis*. This group is paler or brighter than the first group and prefers to live indoor [16]. The mosquitoes found in Nginden village belong to the second group although they lay eggs in outdoor breeding sites. While Thongrungkiat et al [17] reported that there are two forms of *Ae. aegypti* adult stage found in Bangkok, Thailand, they are dark form and pale form. The dark form has white scales on the dorsal part of the first abdominal segment. The pale form has white scales on the dorsal part of first to seventh abdominal segments. The dark form was similar to morphotype 1 and the pale form was similar to morphotype 7 found in Surabaya. Tsuda et al [7] found similar pattern of morphotype variation in different village of Petemon village in Sawahan district, Surabaya city during March, April and May. While during July, August and October morphotype 7 was fewer, that that found

in March, April and May then followed by mophorype 5, 6 and 2. However, morphotype 1 was always found greatest during both course of survey. Mogi et al [18,19] found similar pattern of *Ae. aegypti* morphotype variation in Phillipine and Thailand that morphotype 1 was predominant among seven morphotypes.

The adult mosquitoes reared from larva collected from indoor of Nginden village showed the similar pattern of morphotype variation to that collected from outdoor. This result indicated that the indoor mosquitoes with pale color also laid eggs in outdoor, because when they are reared to be an adult mosquito showed the similar pattern of morphotype of adult mosquitoes reared from eggs found in indoor. Similar results were also found in Sawahan and Tandes district of Surabaya city [7] and in Bangkok, Thailand [17]. This study reported that there are two forms of *Ae. aegypti* adult stage, they are dark form and pale form.

CONCLUSION

The seven morphotypes described by Mc Clelland (1974) were also found in Nginden village, Surabaya city, Indonesia. Interesting pattern has been found that morphotype 1 was the highest number and followed by morphotype 7. This pattern was always found in every month of collection time. Therefore, the similar study is good to be done during dry season to find out the pattern of morphotype distribution of *Ae. aegypti.* Furthermore, the correlation of morphotype with DHF transmission, seasonal distribution of *Ae.agypti* and other factors are needed to be investigated.

REFERENCES

1. Sugiyanto S. 2003. Demam berdarah dengue, Tinjauan dan Temuan Baru di Era 2003: pp 119.
2. Aryati. 2006.Epidemiologi molekular virus dengue di Indonesia. Disertai doctor. Program Pasca Sajana Universitas Airlangga Surabaya.
3. Hopp MJ and Foley JA. Worldwide fluctuation in dengue feve cases related to climate variability. Clin. Res, 25:85-94
4. Sumarmo. 1987. Dengue haemorrhagic fever in Indonesia. Southeast Asian J Trop Med Public Health, 18 (3): 45-51.
5. Mattingly PF. 1957. Genetical aspect of the *Aedes aegypti* problems. I. Taxonomy and Bionomics. Ann. Trop Med Parasitol, 51: 392-405.
6. McClelland GAH. 1974. A worldwide survey of varian in scale pattern of the abdominal tergum of *Aedes aegypti* (L) (Diptera: Culicidae). Trans R Ent Soc. Land, 126 (2): 239-259.
7. Tsuda Y, Yotopranoto S, Bensryman SS, Rosmanida, Dachlan YP and Takagi M. 2003. Seasonal changes in variation of dorsal scale pattern of *Aedes aegypti* (L.) (Diptera: Culicidae) in Surabaya, Indonesia. Med Entomol Zool, 54 (1): 73-80.
8. Departemen Kesehatan Republik Indonesia, 1989. Kunci Identifikasi *Aedes*, jentik dan dewasa di Jawa. Depkes RI. Jakarta.
9. Hasyimi M dan Sukirno M. 2004. Pengamatan tempat perindukan *Aedes aegypti* pada tempat penampungan air rumah tangga pada masyarakat pengguna air olahan. Jurnal Ekologi Kesehatan. 13 (1): 37-42

10. Yotopranoto s, Rosmanida, Bendryman SS. 2003. Analisi morfotipe *Aedes aegypti* (L.) (Diptera: Culicidae) di Kecamatan Sawahan dan Tandes, Kota Surabaya. Majalah Kedokteran tropis Indonesia, 14 (3): 47-56.
11. Gionar, YR., Rusmiarto, S dan Susapto. 2001. Sumur Sebagai Habitat yang Penting untuk Perkembangbiakan Nyamuk *Aedes aegypti* L. Bulletin Penelitian Kesehatan. 29(1): 21-23.
12. Tran VP and Vu SN. 1999.Key breeding sites of dengue vectors in Hanoi, Vietnam 1994-1997. Dengue Bulletin, 23: 1-4.
13. Preechaporn W, Jaroensutasinee and Jaroensutasinee K. 2007. Seasonal prevalence of *Aedes aegypti* and *Aedes albopictus* in three topographical areas of Southern Thailand. World Academy of Science, Engineering and Technology, 36: 23-27.
14. Montgomery BL and Ritchie SA. 2002. Roof gutters: a key container for *Aedes aegypti* and *Ochlerotatus notocriptus* (Diptera: ulicidae) in Australia. Am J Trop Med Hyg, 67 (3): 244-246.
15. Yotopranoto S, Kusmartisnawati, Machfudz, Arwati H, Sofaria A, Suwandito, Rehatta RM. 2007. Vektor demam berdarah dengue di Kecamatan Tambaksari, Surabaya. Majalah Kedokteran Tropis Indonesia, 18 (3): 62-68.
16. Mac Clelland, GAH. 1975. A Worldwide Survey of Variation in Scale Pattern of the Abdominal Tergum of *Aedes aegypti* (L) (Diptera, Culicidae). Trans. R. Ent Soc Land. 126 (2): 239-259.
17. Thong Rungkiat S, Wasinpiyamongkol L, Maneekan P, Prumongkol S and Samung Y. 2012. Natural Transovarial Dengue Virus Infection Rate in Both Sexes of Dark and Pale Forms of *Aedes aegypti* From an Urban Area of Bangkok, Thailand. Southest Asian J Trop Med Public Health, 43 (5): 1146-1152.
18. Mogi M, Choochote W, Okazawa T, Khamboonruang C and Suwanpanit P, 1989. Scale Pattern Variantions of *Ae. aegypti* in Chiang Mai, Northern Thailand. J Am Mosq Contr Assoc, 5(4): 529-533.
19. Mogi M Okazawa T, Miyagi I and De Las Llagas LA, 1984. Variation in Abdominal Colour Pattern in Eight Populations of *Ae. aegypti* from the Philippines. Mosq News, 44 (1): 60-65.

ANALYSIS OFPESTICIDES LEVELS ONSOME USUAL FRESHVEGETABLESCONSUMEDINMEDAN 2013

Evawani Martalena Silitonga
Department of Environmental Health, Public Health, STIKes Sumatera Utara, Medan
Correspondence: evawanisilitonga@gmail.com

ABSTRACT

Background: The presence of pesticides increases agricultural production and farmers' welfare. On the other hand, excessive use of pesticides may endanger human health and environment. Pesticide may also contaminate vegetables. The purpose of this research was: 1. to describe pesticide levels and 2. to determine the content of existing pesticide in fresh vegetables commonly consumed by people in Medan.
Method: The study was a descriptive survey conducted. Examination of pesticide in vegetables was performed in BTKL Laboratory, Medan. Vegetables examined were spinach, chicory, pahir vegetables, kale, and cabbage. Analysis of the study was based on Regulation of Agriculture Ministry No. 24/Permentan/SR.140/4/2011. The examination was to detect residual organophospat in vegetables. The threshold of pesticide levels consisted of: normal (<0.05mg/kg), exceeding the normal threshold (>0.05mg/kg) and permissible tolerance limit (0.10ppm).
Results: The results showed that all the vegetable samples were negative in residual pesticide. Some vegetables contained pesticides, but the residual levels were below the set threshold value. The entire content of pesticides vegetables studied were normal.
Conclusion: Vegetables in Medan have low or negative contamination of pesticide. However, farmers are expected to use pesticides in accordance with the recommended rules to ensure the safety of vegetables produced. Besides, people should to wash the vegetables that will be consumed with running water.
Keywords: vegetables, pesticide

BACKGROUND

Pesticides are chemical substances used to control various pests, such as insects, mites, fungi, bacteria, viruses, nematodes, snails, mice, birds and other animals are considered harmful. Pesticides are also defined as chemical substances and other substances that regulate and or stimulate the growth of plants or plant parts. According to the concept of Integrated Pest Management (IPM), pesticide use is not intended to eradicate or kill pests, but more to control pests such an extent that under the economic threshold or threshold control (Djojosumarto, 2008).

Based on FAO data, pesticide use can save 50 percent on the results of the cotton plant. The role of pesticides is very large and is an important tool that is indispensable in the field of agriculture. Agricultural intensification is performed by applying a variety of advanced technologies such as the use of fertilizer, high yielding varieties, improved irrigation and cropping patterns. These will lead to changes in the ecosystem that is often followed by increased body attacks bully problem. Similarly, effort of agricultural extension in order to open up new agricultural land, which means to revamp the ecosystem, often followed by the onset of an attack remains a bully problem.

In Indonesia, for the purposes of crop protection, particularly for agriculture and forestry, 1702 pesticide formulations had registered and permitted to use in 2008. However, in integrated control of crop pests, pesticides should be the last alternative. Furthermore, government no longer subsidizes the pesticide. But in reality many farmers still use it. Responding to this, government and private sector must constantly providing information about how to use pesticides safely and properly. Safely is considered as safe for pesticide users and their surroundings, while proper is considered as proper use of pesticide type, application method, target, time, and dose (Wudiyanto, 1990).

The number of samples tested each year between 60-90 samples per year. And the results of the sample test conducted in general laboratory results showed that the content of pesticide residues still below the maximum residue limit (MRL), which means it is still suitable for consumption. However there are some commodities that need special attention and need to be developed by the district/city such as the use of pesticides is good and true in the commodity red pepper and tomato, which is quite risky commodity on the season change, so the use of pesticides is quite high (DAILY ANALISA, 2010). Many pesticide residues pose variety of health problems, both in long and short term. For example, pesticide may inhibit cognitive development, cause congenital abnormalities, which shoud be avoided. Pesticide residual can be found in fresh fruits and vegetables, so we need caution in taking it.

Sambu Central Market is one of the largest shopping mall located in the city of Medan. All vegetables are not produced from the field but delivered from several areas in the agricultural area. No study has been done to examine pesticide levels in the fresh vegetables. In this study we analyzed pesticide levels in some fresh vegetables in Medan.

METHOD

Design, Location and Time

The study was a descriptive survey is to see an overview of pesticide levels in some fresh vegetable consumption is common in the city of Medan in 2013. Study site is in the city's central shopping center field, as for the reason for the choice of location is: (a) Medan is a metropolitan city where one of the said items of income from rural to urban; (b) The city of Medan consumes more vegetables that have been sold in the market center. Study was conducted in February 2013 until March of 2013.

Object

Research object is fresh vegetables such as spinach, cabbage, kale vegetable, vegetable trenches, chicory and mustard bitter, people who consumed the city of Medan

Method of Measurement and Analysis of Pesticide Levels in Vegetables

How to do a pesticide on fresh vegetables, performed by some representatives, namely: (a) Areas that really appears to have used pesticides intensively; (b) Overlays and plot the existing plant at the time; (c) Commodity to be analyzed; (d) Pesticides in use; (e) Sampling time (Directorate of plant protection, 2000).

Of some of the above ways, the way of the examination of pesticides on fresh vegetables on the way researchers use point to 3 as follows: Commodity to be analyzed

before taking samples for analysis vegetables, need to pay attention to the tools and materials to be used in sampling, number, giving labels , delivery and storage of samples. (Directorate of plant protection, 2000)

a. Tools and materials
   The tools used in the dry state, clean, does not leak, does not react with the sample material, is not contaminated with pesticides or other materials or can be closed with a tight belt or be in a meeting.
b. Method of sampling
   Plants / crops retrieval method, randomly selected and specify its location, and the plants were taken at random from the plant stack.

Tabel 1. MinimumNumber ofSamplesI Fresh Vegetables

MaterialWeight(Kg) In theStacks	Minimumweight(kg) samples
<50	3
50-500	5
500-2000	10
>2000	15

(Direktorat Perlindungan Tanaman, 2000/ Directorate of Plant Protection, 2000))

Tabel 2. Number ofSamplesExaminedInFresh Vegetablesinthe laboratory

MaterialWeight(Kg) In theStacks	Minimumweight(kg) samples
< 20	1,5
25-250	3
250-1000	5
>1000	7

Sample size that was taken depends on the type and size of the stem and the large leaves and the large container and then the container is using that then the method of analysis and the results desired. Intake of vegetables is better taken at the recently completed harvest vegetables.

Pesticide levels calculation using analytical methods multiresidue Organophosphate pesticides in nonfat matrix (sun method in the commission of pesticides Department of Agriculture, 1997) REGULATION OF THE MINISTER OF AGRICULTURE No.: 24/Permentan/SR.140/4/2011. This method is used in the determination of pesticide residues of acephate, azinfosetil, carbofenotion, koumafos and vegetables as well as fruits, value recovery in this method is greater than 80% with a limit of determination from 0.01 to 0.05 mg / kg. The data contained in the pesticide content of fresh vegetables consumed was obtained by inspecting the lab and the results compared with a threshold value according to Minister of pesticides on vegetables.

RESULT

Based on laboratory tests conducted, the obtained results of measurements of pesticide content in some fresh vegetables are as follows:

Tabel 3. Distribution ofPesticide ResidueTesting Results

No	Vegetables	Propenofos	Dimetoat	Klorpirifos	Fention
1.	Spinach	-	-	-	-
2.	Chicory	-	-	-	-
3.	Bitter mustard	-	-	-	-
4.	Kangkung	-	-	-	-
5	Cabbage	-	-	-	-
6	Trenches vegetable	-	-	-	-

DISCUSSION

Eligibility of pesticide testing

a. Pesticide testing was on leaf and stem.
b. Deliveries were conducted immediately after the sample was taken to minimize contamination and residual loss. Our results were then expected to be completely accurate.
c. All vegetables was Karo origin.
d. All samples were negative of organophosphate pesticide, which means that all the samples tested did not contain residues of organophosphate pesticides.
e. All samples were negative of propenofos pesticide, which means that all the samples tested did not contain residues of organophosphate pesticides.
f. All samples were negative of dimethote pesticide, which means that all the samples tested did not contain residues of organophosphate pesticides.
g. All samples were negative of chlorpyrifos pesticide, which means that all the samples tested did not contain residues of organophosphate pesticides.
h. All samples were negative of fention pesticide, which means that all the samples tested did not contain residues of organophosphate pesticides.
i. Thus, all vegetables are safe for consumption

Vegetables is important in human life because they contains minerals and vitamins that humans need. However, vegetable consumption by the public is still below the requirement due to level of knowledge, low productivity of vegetables etc. Factors limiting productivity are various crop pests and post harvest handling problems that can degrade the quality kuantias and vegetables. One attempt to increase productivity is using pesticide.

Our study showed vegetables in Medan were safe to consume. Low levels of pesticide residues in food will not cause the symptoms of acute or chronic poisoning, although succesive pesticide in low dose may cause subtle effects. Effects can be subtle and pathological changes hystology, carcinogenic, tumorigenic, mutagenic and teratogenic. For example, cytologic changes may occur in the 5-15 ppm DDT in the food ration of male rats. These changes are reversible, suggesting the existence of " induction " of the enzyme in the liver (Ortega, 1962). Organophosphate and carbamate insecticides may cause effects on the neuropathology due to demyelination of nerve tissue protector.

Pesticide residual can be reduced by: (a). Selection of insectice type that effective against pests, safe for humans and environmental, and have low residual; (b). The use and development of new insecticides that more specific and safer such as biological insecticides, insect Growth Regulator, attractant and others; (c). Appropriate dosage and how to use the right application in accordance with the recommendation; and (d). Reduced the frequency of pesticides spraying only when necessary, i.e. when the pest population level that exceeds the level of economic disadvantage. Post-harvest handling can be done to reduce pesticide residues, among others: washing, paring, soaking in hot water (blanching), concoction, and processing in the industry, such as canning, also reduced pesticide residues.

CONCLUSION

Our study concluded: (a) The Market Center is a bustling market and many people visit a lot of different places; (b) Community shopping center and market average worker does not have fields for vegetable gardening; (c) Kale vegetable samples from the center of the market do not contain pesticide residues from qualitative testing using Thin Layer Chromatography method; (d) Chicory vegetable samples did not contain residues of organophosphate pesticides qualitative testing using Thin Layer Chromatography method; (d) Bitter herbs and spinach samples both from the ground Berastagi karo, mustard Thus it is possible that the existing content of pesticides sold and after being tested negative.

REFERENCES

1. Department of Agriculture, 1997) REGULATION OF THE MINISTER OF AGRICULTURE No.: 24/Permentan/SR.140/4/2011.
2. http://www.okefood.com/read/2013/04/22/304/795819/cara-mudah-singkirkan-pestisida-pada-buah-sayuran
3. http://www.merdeka.com/sehat/15-buah-dan-sayur-non-organik-yang-bebas-pestisida.html
4. Yusnani1, Anwar Daud2, Anwar2, IDENTIFICATION OF PESTICIDE RESIDUES ON POTATO ORGANOFOSFAT GROUPS IN THE LOTTEMART AND TERONG MARKET OF MAKASSAR 2013. http://repository.unhas.ac.id/bitstream/handle/123456789/4595/YUSNANI_K11111622.pdf?sequence=1
5. D. Mutiatikum, Puji Lestari S, dan Alegantina, 2002. Analisis Residu Pestisida Piretrin Dalam tomat dan Selada dari Beberapa Pasar di Jakarta. Media Litbangkes Vol. XII No. 2 Tahun 2002. http://ejournal.litbang.depkes.go.id

EPIDEMIOLOGY OF PESTICIDE POISONING ON VEGETABLE FARMER (A REVIEW OF PESTICIDE STUDIES IN KARO DISTRICT)

Donal Nababan
Department of Epidemiology, Public Health, STIKes Sumatera Utara, Medan
Correspondence: nababan_donal@yahoo.com

ABSTRACT

Background: Pesticides are substances meant for preventing, destroying or mitigating any pest. Pesticides may cause acute and delayed health effects in workers who are exposed.
Objective: The purpose of this article is to provide an overview of pesticide poisoning in Karo District and the relevant epidemiologic characteristics.
Data Source: Most of the literatures used as reference in these review were journal and previous academic research results (i.e. thesis and final academic paper).
Results: Characteristic of farmer majority were men, junior high school, age 30-39 years, long term of using pesticide > 10 years, daily long term off spraying > 4 hours, using more than1 kind of pesticide, daily working hours 2-3 hours, duration of working 5-10 years, senior high school, related to eyes health, frequency of spraying were once in 10 days. The pesticide impact to human were health complaint such as vomiting and salivating, suffered poisoning, Acetil Cholinesterase, simple skin irritation, affecting the nervous system, mimicking hormones causing reproductive problems. And the impact to environment were deposits of organochlor pesticide residues in soil, and clean water containing nitrate. Factor related to the pesticide poisoning were young age (RP=1.86), low education (RP=2,52), duration of exposure 2 hours (RP=2,23) wearing PPE is not very good (RP=1,69), age, knowledge, farmer habit, daily spraying and time of handling, habitual (OR 3,121), lifetime days of pesticide application (OR = 1.74; 95%). Intervention is needed against factors related to pesticide poisoning in karo district by Managing pesticide and spraying time, promoting and providing information, and improving the quality of the surveillance data.
Conclusion: Strong evidence exists for impact of pesticide exposure including human health and environment. Monitoring and intervention are needed to be given continuously by competent authority in counseling and guiding how to handle pesticide correctly to avoid pesticide exposure in the farmer activitiy.
Keywords: epidemiology, pesticides, poisoning, karo district

BACKGROUND

A pesticide is a substances primarily used to prevent and destroy agricultural pests. Some of these pests that people do not want are insects, microbes that destroy plants, and other things that affect humans in a bad way and it may kill humans too. Pesticides are included in scientific and technological developments. They are beneficial in such that they control insect infestation and increase agricultural production. However, they are designed to destroy living organisms and therefore also create hazards for human and animal health, and the environment. Synthetic pesticides

have occupied a significant place among the more than 70,000 chemical substances available on the market and they have become the principal strategy for the control of pests.

Kinds of Poisoning were (a) acute poisoning is the severe poisoning which occurs after exposure to a single dose of pesticide. (b) Chronic poisoning is the poisoning which occurs as a result of repeated, small, non-lethal doses over a long period of time with symptoms may appear, such as nervousness, slowed reflexes, irritability, or a general decline in health. General Symptoms of pesticide poisoning were (1) Mild Poisoning such as headache, weakness, nervousness, nausea, diarrhea, loss of weight, thirst, moodiness, skin irritation, eye irritation, etc. (2) Moderate Poisoning such as nausea, diarrhea, excessive saliva, stomach cramps, difficulty in breathing, etc. (3) Severe Poisoning such as fever, intense thirst, increased rate of breathing, vomiting, inability to breathe, etc.

The causes of pesticide poisoning presented are based classification, which depends on the circumstance of exposure: (a) Occupational, Exposure to pesticides during work (Reeves, 2003. (b) Accidental, Exposure to pesticides that occurs unexpectedly (WHO, 2004). (c) Intentional, Exposure to pesticides with intent to cause harm (Calvert, G. M, 2006). It is estimated that about 3% of exposed agricultural workers suffer from an episode of acute pesticide poisoning (APP) every year. More than 50% of all APPs occur in less industrialized countries, though the quantity of pesticides used is less. This illustrates the deficient hygiene and safety conditions under which these products are used. In addition to the acute effects, prolonged periods of low level exposure to pesticides can also produce chronic effects such as damages to the central nervous system, congenital malformations, mutagenic effects, cancer, skin, lungs and eye lesions, damage to the immune system, and masculine sterility, among others.

In some of countries there has been a constant increase in the use of pesticides. Unfortunately, this increase was accompanied by inappropriate use of the products, deficient storage and production conditions, a lack of understanding of the real health effects due to unspecific symptomatology, and a lack of research on the long term effects of these products on health and environmental deterioration. However, underreporting is still considerable due to difficulties of farm workers access to health services, erroneous diagnoses, and problems in registration and reporting.

Many pesticides are poisonous and bad for for humans. A pesticide also affects the environment and the atmosphere. The WHO and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die. To evaluate the epidemiology and characteristics of acute poisoning, it retrospectively analyzed the data of 2867 patients with poisoning who had been treated at the Emergency Center, Fujian Provincial Hospital, China from January 2004 to December 2009. The incidence of poisoning was in a descending order: alcohol poisoning, medication poisoning, and pesticide poisoning (Feng Chen, Jun-ping Wen, Xiao-ping Wang, Qing-ming Lin, Cai-jing Lin, 2010).

Farmers in karo district are the most user of pesticide in national level. Thus conveyed Plt Kadis farms and plantations of Karo Regency, Noumi Sinuhaji. He expects that the paradigm of traditional agriculture in Karo district need immediate

changed from chemical usage being organic (Harian Waspada OnLine, tanggal 17 Juni 2009)

METHOD

The purpose of this article is to provide an overview of pesticide poisoning in Karo District and the relevant epidemiologic characteristics. The data was obtained from student's research which is published on the internet. Most of the literatures used in these review were journals and previous academic research results (i.e. thesis and final academic papers).

Based on these data, the trend of pesticide poisoning was analyzed; further, the effect of pesticide for human health and environment. The data on the number of cases of pesticide poisoning was used for the epidemiological analyses. These epidemiological data were stratified by sex, age, and cause of pesticide poisoning. Proportional analyses were done for each journal and for the other source. The statistical analysis is showing trends of the epidemiological of pesticide poisoning.

RESULT AND DISCUSSION

Based on the review of the literature of using pesticide in Karo district, the results are:

The impact of Using Pesticide

The impact of pesticide use on human health can be known from the following research results:

1. Human Health Effect

The descriptive study about characteristic and personal hygiene of horticultura farmer and health complaint in using of pesticide was reported that majority was men, junior high school, age 30-39 years, long term of using pesticide > 10 years, daily long term off spraying > 4 hours, using more than1 kind of pesticide, Good personal Hygiene because change working clothe and bathing with soap (78,57%), washing hands before lunch, drink, and smoke (100%), but sometime have complaint with their health such as vomiting and salivating (Meliala, 2006). Another study was reported that 21,7% of 23 woman farmer was suffered poisoning (Meliala, 2012).

The study with cross-sectional design and the sample were 60 people through interviews and examination of blood. This study showed that the entire power sprayers in PT Bibit Baru in Dolat Rakyat sub district had Acetil Cholinesterase enzyme activity below normal. Long time spraying factor, spraying time lag and spraying method significantly associated with enzyme activity levels Acetil Cholinesterase (Sitepu, 2010).

Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer. A 2007 systematic review found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure". Strong evidence also exists for other negative outcomes from pesticide exposure including neurological,

birth defects, fetal death, and neuro-developmental disorder (Jurewicz J, Hanke W., 2008).

A 2007 study by the California Department of Public Health found that women in the first eight weeks of pregnancy who live near farm fields sprayed with the organochlorine pesticides dicofol and endosulfan are several times more likely to give birth to children with autism (Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. 2007)

2. Environment Effect

The impact of pesticide use on environment can be known from the following research results: The study reported that there are deposits of organochlor pesticide residues in soil. The levels of pesticide residues are found the most numerous is DDT, either on a sample I and II (Simanullang, 2010). And study in Surbakti village Simpang Empat Karo district reported that the kind of pesticide was Organofosfat (Dursban, Curacron, Perfecthion, Orthene, Lebaycid, etc) and carbamate (Manik, 2012). Another study reported that the clean water found containing nitrate but still qualified health because it still under set the maximum limit of 10 mg/l (Ginting, 2007).

To investigate the pesticide residues level, samples of citrus fruit were collected from citrus field operated by trained farmers as well as from untrained farmers. Research shows that the biodiversity level of soil arthropod on the citrus fields operated by trained farmers is higher than on untrained farmers. The pesticide residue analysis shows that no pesticide residues found on the fruit samples collected from the trained farmers. Unlikely, the residue of Fenvalerate (0,0928mg/kg) is detected on the fruit samples of untrained farmers, although the level is under the Residual Maximum Limit (Wita, 2013).

In order to reduce negative impacts, pesticides need to be deactivated in the environment. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination (Miller, 2004).

Faktor related to the pesticide poisoning

Pesticide management is in activity including the purchase, transportation, storage, dilution, use and disposal of pesticide in Berastepu village, Simpang Empat sub-district, Karo district in 2011. This study was a descriptive survey. The data was taken from the interview and observation on the community of pesticide users (orange fruit farmers) in Berastepu village, Simpang Empat sub-district, Karo regency. The population of orange fruit farmers were 100 families and the sample was taken for 50 orange fruit farmers with simple random sampling. The data used was primary data by distributing the questionnaire and the secondary data. The objective of this research was to know the pattern and behavior of orange fruit farmers, pesticide spraying and its impact on the society health in Berastepu village, Simpang Empat sub-district, Karo district in 2011. The results of the research showed that mostly the characteristics of the respondents were aged between 31-39 years old (38%), working hours in the application of pesticide was between 2-3 hours/day (82%), duration of working in the application of

pesticide was between 5-10 years (50%), education level with senior high school (46%). The behavior in the management was with moderate knowledge (94%), moderate attitude (74%), moderate practice (80%). Mostly the complaint were related to eyes health (32%), and mostly the frequency of spraying were once in 10 days (42%). The conclusion from the research can be taken, that is orange fruit farmers had moderate knowledge, moderate attitude, and moderate practice in the management of pesticide and the frequency of spraying was generally good (Sitepu, 2012).

Pesticide is a chemical compund of toxic and harmful material. If it s not carried out well, it can induce negative impacts for human health and the environtment. Prevalance proportion of pesticide poisoning on citrus farmers spray in Perteguhen village, Simpang Empat sub-district is 57.13%. This research aims to know the factors related with the pesticides poisoning issues on citrus farmers spray in Cinta Rakyat village, Merdeka sub-district on 2010. This research is an analytic studies with cross-sectional design method. The populate is all of citrus farmers average ages 19-60 years. The sample is 120 person by using purpossive sampling by a direct interviewing using closed- questionnaires, univariate-bivariate data analysis, and chi-square test. From the result, it was founded that prevalance proportion of pesticide poisoning in Cinta Rakyat village, Merdeka sub-district 36.7%. The result with chi-square test, find that a significant relationship association. They are, young age (RP=1.86; p=0.00); low education (RP= 2,52 ; p=0.00); duration of exposure 2 hours (RP= 2,23; p=0.00), wearing protective equipments and is not very good (RP=1,69; p=0.03) for pesticides poisoning event (Ginting, 2011).

The research's objective is to find out the poisoning condition description of pesticide user on vegetable farmer and related factor to the poisoning incident. This is an analytic descriptive study, using observational method by case control research design with 134 farmer as case and 134 farmer as control. Bivariat test result using chi-square test show that several significant variabel are age, farmer habit, apply of personal protective equipment (PPE), daily spraying, and time of handling with poisoning incident. The most related variable is habitual (OR of 3,121). Interaction test show relationship of PPE application with poisoning incident give different effect for those with old and young of age. Poisoning gives the different effect for those who applying completeness and incompleteness of PPE. The objective of this study was to explore work-related risk factors of acute occupational pesticide poisoning among male farmers according to the severity of the poisoning. A nationwide sampling survey of male farmers was conducted in South Korea in 2011. A total of 1,958 male farmers were interviewed. Severity of occupational pesticide poisoning in 2010 was evaluated according to symptoms, types of treatment, and number of pesticide poisoning incidents per individual. A multinomial logistic regression model was used to estimate the odds ratio with 95% confidence intervals for risk factors of acute occupational pesticide poisoning. We found that the risk of acute occupational pesticide poisoning increased with lifetime days of pesticide application (OR = 1.74; 95% CI = 1.32–2.29), working a farm of three or more acres in size (OR = 1.49), not wearing personal protective equipment such as gloves (OR = 1.29) or masks (OR = 1.39). Those who engaged in inappropriate work behaviors such as not following pesticide label instructions (OR = 1.61), applying the pesticide in full sun (OR = 1.48), and applying the pesticide upwind (OR = 1.54) had a significantly increased risk of pesticide poisoning. There was no significant risk difference by type of farming. In addition, the magnitude of these risk

factors did not differ significantly by severity of acute pesticide poisoning. In fact, our findings suggest that work-related risk factors contributed to the development of acute occupational pesticide poisoning without relation to its severity (Ji-Hyun Kim, Jaeyoung Kim, Eun Shil Cha, Yousun Ko, Doo Hwan Kim and Won Jin Lee**,** 2013)**.**

Prevention and control of pesticide poisoning

Farmer's knowledge and attitudes were important factors that influenced the practice of farmers in the used of pesticides. Farmers who have the knowledge and good attitude were expected to have a good action was also in the use of pesticides. This type of research was Quasi Experiment with the design of "Separate sample pretest-posttest", treatment were the provision of counseling with lectures and distribution of leaflets. The samples were 40 people. The sample was divided into two groups: the intervention group and control group numbers 20 people respectively. Analysis of the results carried out using paired sample t-test. This research purpose to know the effected of of pesticides counseling on knowledge and attitudes in citrus farmers spray pesticides in Serdang village Barusjahe Sub-distrik,Karo Distrik 2011. Results showed before the counseling citrus farmers have good knowledge in the intervention group and control group respectively 65% and 35% were knowledgeable. After counseling knowledge citrus farmers in the intervention group became both 100% and in the control group still has a good knowledge of 65% and 35% were knowledge able. The attitude of citrus farmers are given counseling before having a good attitude in the intervention group and control group respectively 85% and have the attitude was 15%. After receiving counseling attitude citrus farmers in the intervention group became both 100% and in the control group to 90% good and 10% had the attitude category (Tarigan, 2012).

The research was held in Perteguhen village Simpang Empat Sub-distric, Karo Distric with the total of the sample were 15 respondents. The result by t-test showed that there was a difference of knowledge and attitude of the pesticide applicators after the pesticide extension (Girsang, 2009)**.** One of shallot producers ini Brebes Regency is Tanjungsari Village, having population of 6.689 inhabitans. An eighty-five percent (85,49%) of its inhabitants is shallot farmer. Considering this condition, the village is the most potential area to produce shallot and the most potential area to endamage environment, due to pesticide use practices by farmers. One of ways to increase Agricultural products is through the effort of pest eradication by using pesticides. Because pesticide is a poisonous substansce causing bad effects on the environment, the user of pesticides needs to handle it well starting from the stages of selection, storage, application, and the discard of pesticide container. The result of Chi-square test shows that factors influencing significantly on pesticide use behavior are: education, knowledge, attitude and peer support. Multiple logistic regression analysis result shows that the most dominant factor influencing on pesticide use behavior is attitude with OR 10,483. It means that respondent having negative attitude on pesticide tend to behave badly 10.483 more than respondents having positive attitude.

After reviewing of literature, there are some recommendation was given by researchers. Pesticide poisoning can be reduced by:

1. Managing spraying time lag and spraying method of pesticides sprayers to reduce pesticide attack (Sitepu, 2010).
2. Paying attention and to have correct pesticide management, especially for those orange fruit farmers in bad category (Sitepu, 2012).

3. Making a counseling about the impacts of using and managing pesticide against risk of pesticide poisoning, do blood-test for those got symptoms of poisoning and increasing of farmers knowledge about the using wearing protective equipments (Ginting, 2011).
4. Health promoting and counseling to increase knowledge and attitude citrus farmers in spraying pesticides. Expected to health workers in health centers for providing information about the use of pesticides to the public to help increase the knowledge of farmers in spraying pesticides. (Tarigan, 2012).
5. Using the group discussion and simulation to intensity the pesticide extension (Girsang, 2009).
6. Socializing personal hygiene for farm workers and their families continually.
7. Improving the quality of the surveillance data for the effective prevention and control activities.

CONCLUSION

Conclusion of this review as follow:

1. Characteristic of farmer majority were men, junior high school, age 30-39 years, long term of using pesticide > 10 years, daily long term off spraying > 4 hours, using more than1 kind of pesticide, daily working hours 2-3 hours, duration of working 5-10 years, senior high school, related to eyes health, frequency of spraying were once in 10 days.
2. The pesticide impact to human were health complaint such as vomiting and salivating, suffered poisoning, Acetil Cholinesterase, simple skin irritation, affecting the nervous system, mimicking hormones causing reproductive problems. And the impact to environment were deposits of organochlor pesticide residues in soil, and clean water containing nitrate.
3. Factor related to the pesticide poisoning were young age (RP=1.86), low education (RP=2,52), duration of exposure 2 hours (RP=2,23) wearing PPE is not very good (RP=1,69), age, knowledge, farmer habit, daily spraying and time of handling, habitual (OR 3,121), lifetime days of pesticide application (OR = 1.74; 95%).
4. Intervention is needed against factors related to pesticide poisoning in karo district by Managing pesticide and spraying time, promoting and providing information, and improving the quality of the surveillance data.
5. Research with literature review is useful to look at several studies simultaneously so that it brings new findings on a particular topic that has been researched.

REFERENCES

Calvert, G. M.; Barnett, M.; Mehler, L. N.; Becker, A.; Das, R.; Beckman, J.; Male, D.; Sievert, J.; Thomsen, C.; Morrissey, B. 2006. "Acute pesticide-related illness among emergency responders, 1993–2002". American Journal of Industrial Medicine 49 (5): 383–393. doi:10.1002/ajim.20286. PMID 16570258.

Feng Chen, Jun-ping Wen, Xiao-ping Wang, Qing-ming Lin, Cai-jing Lin, 2010, Epidemiology and characteristics of acute poisoning treated at an emergency center, World J Emerg Med 2010;1(2):154-156.

Ginting, M., 2007, *Analisa Kandungan Pestisida dan Nitrat Pada Penyediaan Air Bersih Desa Cinta Rakyat Kecamatan Simpang Empat Kabupaten Karo Tahun 2006*, *http://repository.usu.ac.id/handle/123456789/38616*).

Ginting, R., 2011, Faktor Yang Berhubungan Dengan Kejadian Keracunan Pestisida Pada Petani Penyemprot Jeruk Di Desa Cinta Rakyat Kecamatan Merdeka Kabupaten Karo Tahun 2010, http:// repository.usu.ac.id/handle/123456789/22988.

Girsang, Julietta, 2010, Pengaruh Penyuluhan Pestisida Terhadap Pengetahuan dan Sikap Penyemprot Pestisida di Desa Perteguhen Kecamatan Simpang Empat Kabupaten Karo Tahun 2009, http://garuda.dikti.go.id/jurnal/detil/id/0:800059/q/pestisida,%20karo/offset/0/limit

Ji-Hyun Kim, Jaeyoung Kim, Eun Shil Cha, Yousun Ko, Doo Hwan Kim and Won Jin Lee, 2013, Work-Related Risk Factors by Severity for Acute Pesticide Poisoning Among Male Farmers in South Korea, International Journal of Environmental Research and Public Health ISSN 1660-4601 www.mdpi.com/journal/ijerph

Jurewicz J, Hanke W (2008). "Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies". Int J Occup Med Environ Health 21 (2): 121–32. doi:10.2478/v10001-008-0014-z. PMID 18614459.)

Manik, T.R., 2013, *Kandungan Pestisida Pada Tanah Dan Jenis-Jenis Pestisida Yang Digunakan (Study Kasus) Di Kecamatan Simpang Empat Kabupaten Karo Provinsi Sumatera Utara*, http:// repository.usu.ac.id/handle/123456789/32813.

Meliala, A., *Karakteristik dan Hygiene Perorangan Petani Hortikultura Serta Keluhan Kesehatan Dalam Penggunaan Pestisida Di Desa Gurukinayan Kecamatan Payung Kabupaten Karo Tahun 2005.*, http://garuda.dikti.go.id/jurnal/detil/id/0:797493/q/pestisida,%20karo/offs/0/limit/15).

Meliala, I. S., 2012, *Strategi Pencegahan Keracunan Pestisida Berdasar Pada Perilaku Petani di Kabupaten Karo*, http://garuda.dikti.go.id/ jurnal/detil/id/0:773767/q/pestisida,%20karo/offset/0/limit/15.

Miller, G.T., 2004, Sustaining the Earth, 6th edition. Thompson Learning, Inc. Pacific Grove, California. Chapter 9, Pages 211-216

Module 9: Symptoms of Pesticide Poisoning, Pesticide Safety Education Program (PSEP) Part of the Pesticide Management Education Program http://psep.cce. cornell.edu/Tutorials/core-tutorial/module09/index.aspx

Reeves, K. S.; Schafer, K. S., 2003. "Greater risks, fewer rights: U.S. Farmworkers and pesticides". International journal of occupational and environmental health 9 (1): 30–39. PMID 12749629.

Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ Health Perspect. 2007;115 (10):1482–9. doi:10.1289/ehp.10168. PMID 17938740. PMC 2022638. Lay summary: EHP, 2007).

Simanulang L.,2012, Analisis Kandungan Residu Pestisida Organochlor Pada Lahan Pertanian Masyarakat Di Kecamatan Barus Jahe Kabupaten Karo Surnatera Utara, http://garuda.dikti.go.id/jurnal/detil/id/0:773952/ q/pestisida,%20karo/offset/0/limit/15).

Sitepu, B.K. 2012, *Pola Dan Perilaku Penyemprotan Pestisida Terhadap Keluhan Kesehatan Petani Jeruk Di Desa Berastepu Kecamatan Simpang Empat Kabupaten Karo Tahun 2011*, http:// repository.usu.ac.id/handle/123456789/30821.

Sitepu, J., 2010, *Analisis Dampak Pestisida Terhadap Kadar Cholinesterase Penyemprot Pestisida Di PT.Bibit Baru Kecamatan Dolat Rakyat Kabupaten Karo Tahun 2009*, http://repository.usu.ac.id/handle/ 123456789/24767.

Suroso, 2002., Factors Related to The Pesticide Poisoning On Vegetable Farmer in Jambi City in The Year of 2001, Post Graduate Study University of Indonesia, Majoring in Rural Health Management School of Public Health, Thesis, Juli 2002.

Tarigan, B., 2012, *Pengaruh Penyuluhan Pestisida Terhadap Pengetahuan Dan Sikap Petani Jeruk Dalam Menyemprot Pestisida Di Desa Serdang Kecamatan Barusjahe Kabupaten Karo Tahun 2011*, *http://repository.usu.ac.id/handle/123456789/24225*.

WHO. (2004) The impact of pesticides on health: preventing intentional and unintentional deaths from pesticide poisoning. 2004: http://www.who.int/mental_health/prevention/suicide/en/PesticidesHealth2.pdf

Wita, K. 2013, *Dampak Penggunaan Pestisida Terhadap Keanekaragaman Arthropoda Tanah Dan Kadar Residu Pestisida Pada Buah Jeruk (Kasus Petani Hortikultura Di Kabupaten Karo)*, http:// repository.usu.ac.id/handle/123456789/16653).

MOSQUITO RESISTANCE TEST TO ORGANOPHOSPHATE INSECTICIDE WITH MICROPLATE TECHNIQUE

Dwi Astuti[1], Gunawan Setiyadi[2]
Indonesian Scientific Resources/LIPI
Correspondence: dwiast_gun@yahoo.com

ABSTRACT

Background: The aim of this research was to identify the resistance of *Aedes aegypti* lavar in Countryside of Gonilan Kartasura Sukoharjo.
Method: This was an observational survey. Sample was taken from positive result of *Aegypti aedes'* egg of installation of ovitrap in all houses in Countryside of Gonilan at the time of activity of surveillance. The egg then is incubated at appliance snare mosquito larva, awaited till reach instar four and test enzimatis use ELISA reader. Data analysis with descriptive analysis which explain the condition of mosquito larva resistance of vector dengue in Countryside of Gonilan.
Results: Result of solution and research indicated that mosquito of *Aedes aegypti* in have resistance to class insecticide of organophosphate though still in low level (AV< 0.07).
Conclusion: Organophosphate remains susceptible to use in study area
Keywords: *Aedes aegypt,* resistance, insecticide

BACKGROUND

The advancement of technology which has come to a molecular era doesn't proceed in parallel with the reduction of the number of disease transmitted by mosquitoes. The epidemic of dengue hemorrhagic fever (DBD) in Indonesia which occurs every year has become a nightmare for the Indonesian society that feels that their health is being threatened. The disease, which is caused by dengue virus, has not until now been able to be cured by any medication. Besides, the society itself doesn't really understand how to eradicate the disease, which can be inferred by the increasing demand on fogging treatment from them. They think that fogging is the only way to exterminate mosquitoes. They feel secured when their houses and environment have been fogged.

However, there are drawbacks associated with the act of fogging the mosquitoes, i.e. it only kills the mature mosquitoes and leaves the larva alive, it contaminate the environment, it can trigger the emergence of new strain of the virus (gene mutation), as well as the development of resistance of the target mosquitoes against insecticides. The trait of resistance is genetically inheritable, which makes the extermination of the new generation difficult. The more effective approach to exterminate mosquitoes is actually the preventive one which focus on the environment improvement, such as the extinction of mosquitoes' habitats by doing three actions included in the 3M slogan (menutup, menguras, menimbun), which consists of the action of covering the water containers, draining the water bath at least once a week, and burying used things that can retain water, such as used tires, cans and bottles.

The village of Gonilan has requested routine fogging to AKL UMS since 2001 until now because the nearest puskesmas (community health center) does not give the fogging service anymore because of the excess request it received. In the early March 2004 the request for fogging was submitted again by the local community after 4 of their people were smitten by hemorrhagic fever. From this information, it can be inferred that the mosquitoes which vector of dengue hemorrhagic fever virus still exist in this area, despite the routine fogging it received every year. The fogging was carried out using 1 liter of 96% malation mixed with 20 liter diesel fuel (the dose of the malation was 4,57%).

The incidence DBD morbidity in the Village of Gonilan Kartasura, which has received organophosphate incecticide (malation) spraying bring about questions and concerns about the possibility of occurance of the vector mosquito resistance (Aedes aegypti) against the incecticide. Moreover, the characteristics of the female mosquitoes which tend to live in the dark spaces in the house and fly in the 50-100 meter distance around the house surmise the possibility that the mosquitoes transmitting the DBD virus are the offspring of the ones experienced malation spraying several years back. With such background, it is necessary to study the resistance of Aedes aegypti mosquitoes against organophosphate insecticide in the Village of Gonilan.

The study that was preceded by a surveillance was conducted using biochemical test technique, which was an enzymatic method developed from the previous technique (i.e. contact bioassay established by WHO since 1979). The test provides a lot of advantages in the detection of insecticide resistance and is simpler for the determination of the mosquitoes' vulnerability individually. Recently, the enzymatic method is the most attractive method which draws attention from many experts and begins utilized in the operational scale, especially against organophosphate group of insecticides. This is related to the big variety of organophosphate insecticides used for to extinct DBD vector mosquitoes, e.g. temefos and malation (Mardihusodo, 2003).

A resistance is an occurrence of immunity inside the mosquitos' body against certain insecticide. Resistance can occurred as a result of some factors, i.e. the insects have enzymatic system that neutralized the insecticide poison, the bed of lipid reside in the body of the insect can absorb the incoming insecticide, the stage of the insects influenced by the insecticide, the generation and complexity of the genes of arthropods. Insecticides that work on every stage of the insect, i.e. the one that able to kill egg, larval, pupal, as well as mature insect, will more readily cause resistance. Insects possessing several generation in one year will more easily develop resistance against insecticides compared to those having only one generation in one year. The more genes that control the resistance ability of the insects against insecticides, the slower the development of resistance. In addition, long time exposure of single type insecticide will also cause resistance (Suroso,1983).

There are two mechanisms of resistance of DBD vector mosquitoes against OP insecticides, i.e. the increase activity of esterase enzymes and insensitiveness of AchE (acetylcholine esterase). The biochemistry test using micro plate technique is called an enzymatic method because it can detect the increase of the activity of esterase enzyme in the instar four larva. At this instar, the enzyme plays the most important role (Widiarti, 2003).

The larvae were taken from the Village of Gonilan by application of malation once a year for 4 years. Study results by Fikrie in Kulon Progo in 1995 and Suwardi in Sampit in 2002 (in Mardihusodo, 2003) which apply temefos (abate) 4 times a year shows that after 4,5 to 5 years half of Aedes eigipty population has become resistant.

Surveilance is an important supporting activity in every disease control program. The aim of surveillance activity is to obtain information about the density and distribution of DBD vector, the potential places for nesting, fly distance and infiltration direction to the society, and the influence of climatic change/mutation to the vector population. The entomologic indicator used for the DBD verctor surveillance are: Container Index, House Index, larva free number, Breteau Index, dan Ovitrap index (Umniyati, 2003).

The aims of this study were: 1) to know whether the Aedes aegypti larvae in Gonilan Kartasura have develop resistance against or are still sensitive to organophosphate insecticides; 2) to know the level of resistance, if any, of the Aedes Aegypti larvae, i.e. whether it is of RR (high resistance), RS (medium resistance) or SS (low resistance); 3) to know the results of DBD vector surveillance conducted in the Village of Gonilan Kartasura Sukoharjo.

The study was expected to benefit as follows: 1) for the community: the community will see to what extent the larvae have develop resistance as a result of their contact with organophosphate pesticide, and they will get awareness that fogging is not the only and best way to exterminate DBD vector mosquito since the subsequent effect of resistance will be more difficult to overcome; 2) for the offices, officers, or technicians functional in the control of vector mosquitoes: the determination of resistance status will be of strategic value in the decision making of the application of insecticides and for the evaluation of the conduction of the control programs. Meanwhile, the surveillance results can be used in the management of the program effectively and efficiently, from the planning (determination of the program targets and strategies), implementation and evaluation.

METHOD

The type of study was observational survey. The study was conducted at the Laboratory of Environmental Chemistry Muhammadiyah University of Surakarta for 10 months.

Sampling technique

The sampling was conducted by taking eggs of *Aedes aegypti* positive results from the installation of ovitrap at all houses in the Gonilan Village during the surveillance period. The eggs were then hatched and left in the larva trap device until they reach instar four (indicated by complete growth of the mosquitoes' hairs). The obtained instar IV larvae were biochemically tested for their resistance using micro plate technique. The number of larvae tested was 25.

Tools and materials

Tools used in the surveilance program were ovitrap, check list, black-painted containers, larva trap, gauze, and the materials were filter paper, and clear water. The tools and materials used in the resistance test were micro plate, reaction tubes, 50µl micropippette, timer, photometer and the and materials were substrate solution (0.5 ml α -naphtyl acetate + acetone 6 g/L + 50 mL phosphate buffer 0.02M pH 7), coupling reagent solution (150 mg fast blue B salt + 15 mL aquadest + 35 mL aquadest + sodium dodecyl sulphate 5% w/v).

Surveillance

Surveillance was conducted by installing ovitrap at every house in the Village of Gonilan for a period of 10 months. The eggs obtained were collected and hatched to obtain larvae for further test of resistance.

Resistance test

1. Every instar IV larvae was ground individually and dissolved in 0.5 mL phosphate buffer solution to for homogenate
2. Aliquot of homogenate (50µl) was transferred into micro plate well in four replications
3. A 50 µL of substrate solution was added to each well. The enzymatic reaction was left to last for 60 seconds.
4. A 50 µL of coupling reagent was added into each well. The color reaction was left to last for 60 seconds. The initially-appeared red color gradually changes into blue color.
5. The biochemistry reaction was stop by addition of 10% acetic acid into each well.
6. The interpretation of the result was conducted as follows:
7. visually (colorimetric) by scoring (0 = colorless, 1 = light blue, 2 = yellowish green, 3 = dark blue; score: 0-2.0 = SS, 2.1-2.5 = RS, 2.6-3 = RR).
8. by reading absorbance value by ELISA reader at 450 nm (AV 0-0.07 = SS, 0.7-0.9 = RS, >0.9 = RR)

Data analysis

Descriptive analysis to the obtained data was carried out to describe the resistance condition of DBD vector mosquitoes in the Village of Gonilan.

RESULT

Surveillance

The distribution of Aedes aegypti mosquito breeding places can be seen at Table 1, the Ovitrap Index (OI), Container Index (CI), House Index (HI) and Larva-free Number (*Angka Bebas Jentik* /ABJ) are presented at Table 2

Table 1. The distribution of Aedes aegypti mosquito breeding places (mosquitoes nests) in Dusun Gonilan, Dusun Tuwak, dan Dusun Keduren of Gonilan Village

Breeding Places	Gonilan			Tuwak			Keduren		
	N	Pos	%	N	Pos	%	N	Pos	%
Bak Mandi (Splash bath)	46	8	5,0	50	10	6,1	26	4	6,1
Bak Air (General water bath)	38	16	10,0	48	6	3,7	22	2	3,0
Tempayan (Water basin)	10	0	0,0	4	2	1,2	4	2	3,0
Padasan (Cistern)	18	6	3,8	34	6	3,7	10	2	3,0
Ember (*Bucket*)	48	2	1,2	28	0	0,0	4	0	0,0
Total	160	32	20,0	164	24	14,7	66	10	15,1

Tabel 2. Ovitrap Index (OI), Container Index (CI), House Index (HI) dan Larva-free Number (*Angka Bebas Jentik/ABJ*)

Parameter (%)	Gonilan	Tuwak	Keduren
OI	39.1	29.5	16.4
CI	20.0	14.6	15.2
HI	50.0	32.1	30.8
ABJ	50.0	67.9	69.2

Note: OI: Ovitrap Index, CI: Container Index, HI: House Index, ABJ: Larva-free Number (Angka Bebas Jentik)

Resistance test

The result of resistance test of *Aedes aegypti* instar IV larvae are presented at Table 3.

Tabel 3. The resistance levels of Aedes aegypti instar IV larvae

No. Sampel	Tingkat Resistensi	No. Sampel	Tingkat Resistensi	No. Sampel	Tingkat Resistensi
1.	0.480	21.	0.330	41.	0.318
2.	0.470	22.	0.399	42.	0.325
3.	0.341	23.	0.434	43.	0.260
4.	0.279	24.	0.324	44.	0.446
5.	0.577	25.	0.434	45.	0.467
6.	0.310	26.	0.373	46.	0.373
7.	0.489	27.	0.411	47.	0.309
8.	0.481	28.	0.324	48.	0.326
9.	0.303	29.	0.543	49.	0.125
10.	0.278	30.	0.174	50.	0.279
11.	0.615	31.	0.445	51.	0.467
12.	0.295	32.	0.384	52.	0.331
13.	0.334	33.	0.443	53.	0.493
14.	0.283	34.	0.321	54.	0.397
15.	0.325	35.	0.469	55.	0.126
16.	0.389	36.	0.168	56.	0.274
17.	0.419	37.	0.235	57.	0.482
18.	0.331	38.	0.429	58.	0.361
19.	0.346	39.	0.463	59.	0.405
20.	0.280	40.	0.371	60.	0.404

DISCUSSION

The surveillance is an activity to obtain information concerning the density and distribution of DBD vector, the potential breeding places, the mosquitoes' fly distance and direction of infiltration into the community, and the influence of climatic changes/mutation to the vector population. The entomological indicators used for the surveillance of DBD vector were Container Index, House Index, Larva-free Number (*Angka Bebas Jentik*), Breteau Index, dan Ovitrap index (Umniyati, 2003).

The results of DBD vector surveillance conducted in Gonilan Village revealed that the breeding places of the mosquitoes were splash bath, general water bath, water basin, cistern, and bucket. The breeding of mosquitoes were mostly found in splash bath and general water bath. The percentages of the breeding places were in the range of 14.7-20%. The assessment of the breeding places as being positive is considered rather unreliable since a place containing a single larva is assessed as positive, just as is the place containing 100 or even 1000 larvae. Whereas, in fact, the potency to cause disease is greater at the place in which the larvae are more populated.

The largest percentage of positive results from the installation of ovitraps was found in Dusun Gonilan, which reach 39.1%; CI ranges from 14.6 to 20%; HI = 30.8-50%. The Larvae-free Number obtained from the calculation (1 – HI) was 50-69.2%. This value of ABJ is still far from the government target, i.e. 95%, hence serious efforts have to be done to increase it.

The DBD vector mosquitoes still exist in the area of Gonilan Village despite the fogging that is done annually. The fogging in this area have been done with 1 liter of 96% malation mixed with 20 liter diesel fuel (malation dosage equal 4.5%). The annual fogging in this area for 4 years has caused *Aedes aegypti* in Gonilan Village resistant to organophosphate insecticides, although the resistance is still in the low level (AV<0.07). Besides, the characteristics of female mosquitos (the blood suckers), which like to stay in the house (in the dark parts) and fly in the distance of 50-100 meter through their entire live imply that the mosquitoes that exist at this time are the offsprings of those exposed to malation spray in the past, several years back. The resistance of insects against insecticides can be thought as the ability of the insect population to withstand the previously lethal insecticide. The resistance observed in this study is a congenital resistance. In the population of the mosquitoes, there have been some individual mosquitoes that are resistant against an insecticide. This trait was inherited by their offspring from a generation to the next, resulting in the existence of an entirely resistant population. Congenital resistance also occurs as a result of genetic mutation. The mutans and their offspring are all resistant. From mechanistic view, congenital resistance is divided into congenital physiologic resistance and congenital behavioral resistance. The congenital physiologic resistances are resulted from some factors, i.e. 1) the slow absorption of insecticide by the insect which causes the insect to stay alive, 2) the storage of insecticide in non-vital tissues of the insects, which leaves the vital tissues unaffected and the insect survive, 3) the fast insecticide excretion ability of the insect, which prevents the concentration of the insecticide from killing the insect, 4) the detoxification of insecticide by enzyme that keep the insect from death.

The congenital behavioral resistance is caused by factors such as 1) change in the habitat of the insect that avoid the insect from being affected by the insecticide. The offspring retain the habitat. And, 2) ”avoidance”, the behavior of the insect to avoid insecticide that prevent them from the insecticide without changing their habitat (Srisasi et al., 1992).

The resistance of vector mosquitoes against insecticides is also triggered by factors that are able to screen mosquitoes subpopulation (Mardihusodo, 2003), i.e.: 1) genetic factors; there are mosquitoes species that are able to be resistant against OP insecticides because of functional genes (can be more than one gene). For example, those related to the increased activity of esterase in the hydrolysis of the insecticide entering the mosquitoes' body; 2) biological factors, which are related to biological aspects, such as generation time or live cycle and behavior. For fast/short generation time, for example, the risk of being resistant against chemical insecticide is bigger. Meanwhile, the behavior of mosquito that

has high mobility causes the mosquito to be able to avoid the application of insecticide; 3) operational factors; they are related to the chemical aspects of the insecticide, such as the formulation and its chemical group which are related to the mechanism of resistance. The application aspects of the insecticide are also of influencing factors, for example in relation with the dosage, the frequency of application yearly, and the duration of the application.

For the biochemistry of the resistance, there are 3 basic mechanisms of physiological resistance (Mardihusodo, 2003), i.e.: 1) the increase of insecticide metabolism in the mosquitos' body by mixed function oxidase, hydrolase, esterase, and glutathion-dependent transferase enzymes; 2) the reduced sensitivity of the target sites in the test mosquitos' body, in the forms of the nerves insensitivity and acetylcholine esterase (AchE) enzyme insensitivity; and 3) reduction of insecticide penetration into the active sites (nerves and AchE).

The use of chemical insecticides in Indonesia and the whole world is still high in different development sectors, such as agriculture and health. From the activity of detection and monitoring, it was concluded that the number and diversity of insect variety indicating resistance phenomena against one or several types or group of pesticide are increasing. Each organism, including *Aedes aegypti* has ability to develop population that are resistant against pesticide. The resistance is indicated by the reduction of the effectiveness of mosquito control using the pesticide.

The development of resistance against pesticide doesn't occur in a short time but it takes place through several generations as a result of continuously repeated treatments with the pesticide. The evolution phenomena can be explained as follows. In the population, there always be particular individuals which are resistant and those which are sensitive. The number or frequency of the resistant individuals is actually lesser than that of the sensitive ones. However, the continuously repeated use of pesticide causes the reduction of the number of the sensitive individuals and leaves behind the resistant ones to survive. The resistant individuals will pair off one another to produce resistant offsprings, which in turn they result in the generation of population dominated by resistant individuals. Every type of insects such as Aedes aegypti mosquitoes is able to retain their traits and pass them to their offsprings in the long time period. Population of insects that has been resistant against one or more types of insecticide will be able to develop resistance against other substances faster, especially if the new substance has the same mechanism of resistance as the previous insecticides. Some factors that influence the rate of resistance development are concentration, frequency and area covered by the spray of pesticide that forms selection pressure in the population of the insects. In the same condition, insect population that receives stronger selection pressure will develop into resistant population in a shorter period than the population receiving weaker selection pressure (Kasumbogo, 2005).

In Indonesia, the intensive use of pesticide malathion and temephos for controlling Aedes aegypti has lasted for more than 25 years. Malathion was registered in 1976, while temephos was in 1974. The use of both types of pesticide in a long term period for the same target has consequently given selection pressure that force the development of resistant Aedes population faster. This occurs especially in places where incidence of endemy was frequently treated by both types of pesticide.

Data from Resistant Pest Management of Michigan State University USA (2003) show that *Aedes aegypti* mosquitoes have been resistant against 16 groups of pesticide in 44 countries, including Indonesia. The resistance of the mosquito against malathion and temephos has been reported to occur in 24 countries. In the countries like Malaysia, *Aedes* mosquitoes have been

reported to be resistant against malathion in 1972 and against temephos in 1976. The resistance against malathion in Thailand and Singapore occurred in 1980 1986 respectively. The report on the resistance of Aedes that has not been recorded in Indonesia doesn't mean that Indonesia is free from pesticide resistance problems. It probably means that the data on Indonesia has not been recorded in the international report since the study on this matter is still limited, or if any, they just have not been reported internationally. However, based on the information from the neighboring countries, concerns that *Aedes* mosquitoes in Indonesia have also been resistant against malathion and temephos in 2004 need to be given attention seriously by all stakeholders.

Aedes mosquitoes belong to the type of insect potential to develop resistant population, which nationally need to be investigated concerning their distribution and intensity of resistance in the area DBD endemy, especially when fogging technique and larvae extinction by abate are still the only reliably applied techniques in controlling DBD vector. Before monitoring of resistance is conducted, it is necessary to determine the standard of detection and monitoring of the resistance in order for the data to be able to be compared. In addition, a strategy in the management of pesticide resistance has to be applied in the Dengue Hemorrhagic Fever Erradication System (Sistem Penanggulangan Penyakit DBD).

The target of the management of pesticide resistance is to slow down, delay and if possible, reverse the resistance development of *Aedes* mosquitoes against pesticides. The management of pesticide resistance is actually not as easy and not as simple as by changing and rotating the types of pesticide used, but many basic scientific aspects have also to be considered, such as population biology, biochemistry of resistance, molecular genetics, statistics, lethal dose calculation, behavior and migration of mosquitoes, cultural systems of the local community, and other basic aspects. Other alternative technologies for controlling DBD vector need to be considered. The development and application of management of pesticide resistance absolutely demand the participation and collaboration among different related scientific disciplines and stakeholders, including different sectors of the central and regional government institutions, pesticide industries and the community in general (Kasumbogo, 2003).

CONCLUSION

This study concluded: (a) *Aedes* mosquitoes in Gonilan Village Kartasura have been resistant against organophosphate insecticide, although the resistance is still in a low level. (AV< 0.07); (b) The surveillance report on the hemorrhagic fever vector conducted in Gonilan Village revealed the breeding places of the mosquitoes to be in splash bath, general water bath, water vessels, cistern and bucket with Larvae-free Number ranges 50 – 69.2%, which is still far from the government target, i.e. 95%; (c) Coninously repeated application of insecticide will lead to an increasing resistance of the mosquitoes.

As for recommendation, we suggested: (a) Pesticide resistance should be given special attention from all stakeholders, including the central and regional governments, pesticide producers and distributors, researchers, academicians, and the community in general; (b) The government needs to make and establish a special policy on the management of pesticide resistance by inter-sector and inter-disciplinary coordination to inhibit, delay or halt the development of insect resistance; (c) In the government policy, the data and mapping of the level severity of the resistance against insecticide should be included.

REFERENCES

Depkes RI.1992. Petunjuk Teknis Pemberantasan Nyamuk Penular penyakit Demam Berdarah Dengue. Jakarta: Depkes.

Dit.Jen. PPM & PLP Dep.Kes. RI. Data kasus DBD per bulan per propinsi tahun 1998 sampai dengan tanggal 9 Mei 1998.

Iskandar, Adang.1985.Pemberantasan Serangga dan Binatang Pengganggu. Jakarta : Depkes RI..

Kasumbogo, U.,2005.Manajemen Resistensi Pestisida sebagai Penerapan Pengelolaan Terpadu.http://kasumbogo.staff.ugm.ac.id/detailarticle=php?mesid=10. Artikel.

Mardihusodo. 2003. Resistensi Insektisida Pada Nyamuk (Makalah Pelatihan). Yogyakarta : Laboratorium Parasitologi UGM.

Srisasi, dkk.,1992. Parasitologi Kedokteran. Jakarta: FK-UI..

Sugiyono. 2QQ3 .Statistika untuk Penelitian. Bandung. CV Alfabeta.

Sumarmo, Suroso T, Abdulkadir A, Lubis I. 1994.The epidemiology, control and prevention of dengue haemorrhagic fever in Indonesia. Cermin Duma Kedokteran 1994; 92:5-10.

Suroso, Thomas. 1983. Tinjauan Keadaan dan Dasar-Dasar Pemikiran dalam Pemberantasan DB di Indonesia Jakarta : Dirjen P3M Depkes RI.

Suroso T,dan Imran A. 1997.A review of dengue haemorrhagic fever and it's control in Indonesia. Presented at the WHO Western Pasific Region/South East Asia Region meeting on prevention and control of dengue/dengue haemorrhagic fever. Manila 14-17 Juli 1997. Makalah.

Umniyati.2003.S'wrve/7/ance (Makalah Pelatihan). Yogyakarta : Laboratorium Parasitologi UGM.

Widiarti.2003.L//7 Peningkatan Aktivitas Enzim Ester ase Non Spesifik menurut Metode Lee (Makalah Pelatihan).Yogyakarta: Laboratorium Parasitologi UGM

Wuryadi S. 1997..Virological surveillance of dengue haemorrhagic fever in Indonesia 1995/1996. Presented at the 4th international symposium on dengue fever. Papeete, Tahiti, 14-17 April 1997. Makalah.

ANOPHELES MOSQUITO SURVEY IN PASEH VILLAGE, BANJARMANGU SUBDISTRICT, BANJARNEGARA

Tutik Ida Rosanti[1], Sunaryo[2]
[1]Department of Parasitology, Medical and Health Science Faculty,
Soedirman University, Purwokerto
[2]Animal-Borne Diseases Unit, Health Research and Development, Banjarnegara
Correspondence: tutikidarosanti@yahoo.com

ABSTRACT

Background: Banjarnegara District is one of malaria endemic district in the Province of Central Java. In period of July 2013, malaria cases had increased; one of them was in Paseh Village, Banjarmangu Subdistrict. Mosquito survey should be conducted in Paseh Village in order to identify the species of *Anopheles* and it's bionomic. Survey data is expected to be useful for mosquito vector control.
Method: This was a rapid survey with cross sectional design. Sample was taken by purposive sampling technique. This study used all night entomology survey with human bait.
Results: The survey recorded that temperature in Paseh Village ranged between 21 and 23°C, humidity ranged between 92% and 99%, with normal wind speed. Three species of *Anopheles* mosquitoes were identified. They were *An. balabacensis*, *An. barbirostris* and *An. Kochi*. *An. balabacensis* and *An. barbirostris* have been confirmed as a vector of malaria in Java. *An. balabacensis* caught mostly on early night near their breeding place, and the rest was on mid-night. *An. barbirostris* mostly caught in the cage. Breeding places were irrigation canals around Salak trees, puddle in Salak garden.
Conclusion: *Anopheles* in study area actively bites in the midnight until morning.
Keywords: malaria, mosquito spot survey, vector control

BACKGROUND

Malaria is disease caused by protozoa of the genus Plasmodium, which remains one of public health problems. The World Helath Organization (WHO) report in 2010 stated that half of world's population had risk of malaria infection by 81 million cases of positive malaria positive and 117,704 died caused by malaria. Malaria in Indonesia is still be major public health problem. Average cases of malaria are estimated about 15 million clinical cases per year. Population at high risk of malaria generally populations that live in malaria endemic areas. Estimation about endemicity low, medium and high level are 85.1 million. Unfortunatelly, 60% of malaria are infecting a lot of the productive ages (MoH, 2007).

Banjarnegara regency is on of the malaria endemic in Central Java province. Almost the whole areas in Banjarnegara regency are malaria-endemic areas. Of 20 districts in Banjarnegara regency, only 3 districts are free from malaria. To change endemicity map many kind of efforts had been done by local goverment in Banjarnegara regency through its District Health Office (DHO). Various effort had been

done to overcome malaria problem in Banjarnegara, such as malaria vectors control, environmental management and treatment of patients. These efforts had showed good result such as diclining in malaria morbidity per thousand population or annual parasite incidence (API). Malaria morbidity during 2001-2008 were 9.07, 15.54, 5.74, 0.77, 0.13, 0.22, 0.37, 0.23 and 0.21 % respectively (DHO Banjarnegara, 2009).

Although malaria morbidity has been declining per thousand population, but the presence of malaria is still continued to exist in Banjarnegara regency. Existance of salak garden in accordance with ditches have made Anopheles mosquitoes as malaria vector remains uncontrolled. Data of Banjarnegara DHO showed in Juli period 2013, there was an increase in cases of malaria, one of which is in Paseh Village, Banjarmangu District. Therefore, it is necessary to surveying species of Anopheles and its bionomic in Paseh Village. The results of this spot survey was expected for effective and efficient mosquito vector control activities.

Geographical conditions Paseh Village, Banjarmangu District, Banjarnegara regency is hilly area, mostly are salak plantation. Water for daily necessary is obtainable from springs channeled into people's houses through pipes. Residents who do not deliver water to his house get water by visitting places of public toilets where the water was also obtained from springs channeled. Paseh river conditions in the village during the dry seaseon or the transition from the rainy season to the dry seaseon raises the hose containing water. The holes can become breeding places of mosquitoes Anopheles sp.

METHOD

This was a spot survey with cross sectional design. A sample is taken by purposive sampling. The whole Anopheles mosquitoes were caught all night long. This study is a collaboration among Banjarnegara DHO, Health Center and Loka Litbang Banjarnegara in Juli 2013 in the Paseh Village, Banjarmangu, Banjarnegara Regency, Central Java.

Tools and materials were a set of tools of entomology survey. This survey form catching mosquitoes all night (all night entomology survey) using 3 people as bait in the house and 3 people outsiede the home. The result catches of mosquitoes every hour identified using identification keys of O'Connor and Arwanty (1979).

RESULT

Table 1 showed species of Anopheles found in Paseh Village, Banjarmangu Subdistrict, Banjarnegara District on 17 Juli 2013.

Table 1. Species and density of Anopheles in Paseh village

No	Species	Density				**Total**
		UOD	UOL	Stall	Wall	
1	*An.balabacencis*	1	0	0	1	2
2	*An.barbirostris*	0	0	7	1	8
3	*An.Kochi*	0	0	0	1	1
Total		1	0	7	3	11

Note: UOD=human bait inside house; UOL=human bait outside house

DISCUSSION

On this spot survey recorded that temperature in the Paseh villages ranges from 21-23°C. Humidity ranged between 92-99% and the windspeed normal. Warm temperatures cause mosquitoes to breed easily and more active sucking blood. 60% humidity levels the lowest limit to allow mosquito life. In a higher humidity mosquitoes become more active and often bite. A spot survey on mosquitoes in the Paseh village, Banjarmangu, Banjarnegara Regency, which caught three species of Anopheles mosquito. Species of mosquitoes found in the Paseh village in the range 06:00 am to 06:00 pm at the time are *An. balabacencis, An. barbirostris, An. kochi*.

An. balabacensis only known a malaria vector in Banjarnegara. *An. balabacencis, An. aconitus* and *An. maculatus* has been confirmed as a vector of malaria in Central Java including Banjarnegara. *An. balabacencis* mosquito in spot survey at this time found at 07.00-08:00 pm only one mosquito in the house. Bionomic of *An. balabacensis* differ from one place to another. This mosquito species is more attracted to human blood both in the home and outside the home and were caught after midnight until early morning at around 04.00 am. Catching *An. balabacensis* mosquitoes at the beginning of the evening indicated that mosquitoes' breeding places has to be around home environment.

Based on our observations, there were many fish in pools with the type of soil that does not drain water and exposed to the sun directly in the vicinity of catching mosquitoes. Naturally, *An. balabacensis* larvae habitat is freshwater puddles in the woods either a permanent or temporary that does not flow as a vehicle ruts or buffalo footprint as well as the banks of a dry river. The location Paseh village that most of its territory there are garden "salak pondoh" generally have a pool that serves for irrigation. At one "pondoh" land also often found the hole stagnant water.

Characteristics such as the presence of areas with high humidity support the 92 to the 99% it has the potential to become a breeding place of Anopheles mosquitoes. The second *An. balabacencis* found on the walls of the house at 11.00-12.00 pm, it could also indicate that behavior *An. balabacencis* after biting mosquitoes cannot go to distant places and choosing wall as a resting place.

An. barbirostrisis is a dominant species found in Paseh village. *An. barbirostris* also bite between 11.00 pm to 05.00 am and after biting they fly down on coffee plantations orpineapple tree, therefore in this survey only one who caught the tail on the wall while the rest were caught in the cage. This mosquito prefers to suck the blood of animals than human blood. Natural habitats in swamps, ponds and irrigated land. This species on the island of Sumatra and Java rare bite people. but on the island of Sulawesi and East Nusa Tenggara many are interested in sucking human blood. Catching of this mosquito in the wall of the house because the cattle pens are close to people's homes even livestock living one house with owner.

CONCLUSION

There are environmental factors such as temperature, humidity, presence of suitable breeding place for mosquitoes *An. balabacensis* existence as a vector of malaria in the Paseh Village Banjarmangu Banjarnegara Regency, then the chances of malaria in this village are very large especially if there are people with malaria case in the

village. Therefore, it should always be done observation and monitoring the existing mosquito larvas in potential places. It needs to think about the use of natural predators mosquito larva in the potential areas. While based on a survey it was found that mosquitoes and *An. barbirostris* and *An. balabacensis* especially caught at midnight until early morning then the locals should be advised to sleep in a mosquito net.

REFERENCES

Barodji. 1987. *Fluktuasi Kepadatan Populasi Vektor Malaria An. aconitus Di Daerah Sekitar Persawahan.* Proc. Seminar Entomologi II, Jakarta

DKK Banjarnegara. *Data Malaria*, Subdin P3PL.Tahun 2009

Gandahusada, S. 2006. *Parasitologi Kedokteran.* Fakultas Kedokteran Universitas Indonesia, Jakarta.

Ikawati,B. 2006. *Balaba*. Potensi Anopheles balabacensis,dahulu dan sekarang. 2 (1) : 18

Pranoto & Prasetyo,P. 1991. Konfirmasi Anopheles balabacensis Baisas sebagai Vektor Malaria dan Anopheles maculatus Theobald sebagai tersangka vektor malaria.

Prasetyo, A. *Malaria.* Jakarta 21 November 2006. From URL: http://.www.Pusat Informasi Penyakit Infeksi khususnya HIV-AIDS – Penyakit – Malaria.html (diakses pada 2 September 2010).

SPATIAL ANALYSIS OF MALARIA DISEASE RISK FACTORS IN THE SIJUNJUNG DISTRICT

Masrizal Dt Mangguang
Department of Epidemiology and Biostatistics, Public Health Faculty, Andalas University
Correspondence: masrizal_khaidir@yahoo.com

ABSTRACT

Background; Malaria is caused by the malaria parasite (Plasmodium) asexual forms into the human body, is transmitted by mosquitoes (Anopheles). Based on the reports of the District Health Office Sijunjung in 2012 there were 94 positive cases of malaria. The purpose of this study was to determine the risk factors related to know the incidence of Malaria and spreading in Sijunjung district in 2013.
Method: This was a case control study. The research was conducted in the District Sijunjung from January to June 2013 with 58 respondents and 58 cases of group control group respondents. Method of sampling in this study is to use a simple random sampling method. Data was processed using Mc Nemar with 95% confidence level (α = 0.05). Multivariate analysis was used logistic regression.
Results: The result showed that the presence of shrubs (OR=2.8 CI=1.208 to 6.756), use of wire netting on ventilation (OR=6.7 CI=2.361 to 19.29), the ceiling (OR=3.5 CI=1.544 to 8.257), the level of knowledge (OR=3.5 CI=1.152 to 10.633), outdoor activities at night (OR=5.3 CI=2.23 to 12.755), the use of mosquito nets (OR=3 CI=1.09 to 8.254) were risk factors associated with the incidence of malaria. As for the existence of the trench is not a risk factor associated with the incidence of malaria. The most dominant risk factor is the presence of the ceiling (OR=5,285 CI=1,592 to 17,392).
Conclusion: The level of knowledge, outdoor activities at night, the use of mosquito nets were risk factors associated with the incidence of malaria. As for the existence of the trench, it was not a risk factor. The most dominant risk factor was the presence of the ceiling. Health workers in the district expected Sijunjung, can improve health promotion activities to the public in order to put gauze on ventilation, use mosquito nets when slept. Avoiding outdoor activities at night, installed the ceiling to reduce the risk of malaria.
Keywords: malaria, risk factor

BACKGROUND

Malaria is one of public health problem that can cause death especially in high-risk groups are infants, toddlers and pregnant woman. Besides malaria directly cause anemia and lowered productivity [1]. According to the World Health Organization (WHO), malaria is widespread in the world. More than 90 countries are infected by this zoonotic disease. Areas with the most cases of malaria are African, America, South Asia, and Southeast Asia. People with malaria in 2011 was 216 million and 655 thousand died, 91% of malaria deaths occur in Africa [2].

In 2010 there were 65% in Indonesian endemic districts, where 45% of the population in the district at risk of contracting malaria. An area of high malaria endemic

categorized or High Incidence Area (HIA) and the incidence of malaria more than 50 cases per 1000 population per year. Malaria-endemic areas are or Medium Incidence Area (MIA) with malaria incidence of 10-50 cases per 1000 population per year and low malaria endemic areas or Low Incidence Area (LIA) and the incidence of malaria is less than 10 cases per 1000 population per year [3,4].

Community survey during 2007-2010, the prevalence of malaria in Indonesia decreased from 1.39-0.6%. Based on reports to the Sub Malaria during the years 2000-2009 have shown a tendency decline in malaria morbidity in a row is 3.62 per 1,000 population in 2000, 1.85 per 1,000 population in 2009 and 1.96 in 2010 with numbers malaria deaths reaching 1.3% [3]. Data from Indonesia Health Profile In 2012, malaria morbidity as measured by Annual Parasite Insidence (API) occurred decline of 2.89% in 2007 to 2.47% in 2008, and an increase of 1.85% in 2009 to 1.96% in 2010, again decreased from 1.75% in 2011 to 1.69% in 2012 [5].

West Sumatra is a province in Indonesia with a population of 4,909,358 souls belonging to endemic malaria. Based on the Health Profile of West Sumatra in 2011, in 2009 obtained the number of malaria cases with as many as 6130 cases of malaria positive cases for 1191 Annual Parasite Incidence or API by 0.41%). In 2010, number of malaria cases was as many as 4701 cases with positive malaria cases by 1104 API Annual Parasite Incidence or 0.11%). In 2011 decreased to 743 cases of malaria positive cases examined 3833 blood test [5,6].

According to the guidelines issued by the Directorate General of Disease Control and Environmental Health (DG PP & PL) in 2012 there were three regencies/cities in West Sumatra Province which has a high incidence rate: Sawah Lunto Town (1.44), Mentawai Islands (1,33), and the District Sijunjung (0,88). Sijunjung district geographically located with hilly topography with altitudes between 100-1500 above sea level (asl), has a total area 3,130 km2 swamps, plantations, has gold mining area since most community livelihood is mining for gold and farmers, a small part again as traders, self-employed, and civil servants. Habits of the people out of the house at night to gather at the stalls. This habit is done by the public until midnight, even until the early hours [7,8].

Based on the reports of the District Health Office Sijunjung in 2011 there were 1,735 cases of clinical malaria by the number of positive malaria cases was 356 cases. While At The 2012 clinical malaria cases decreased to 1154 the number of positive malaria cases 94 people. At some district in Sijunjung district malaria case detection increased significantly positive as Holy Sumpur district. If seen from the figure Annual Parasite Incidence (API) that is positive malaria cases during the year in an area per 1000 population, figures API Sijunjung district in 2012 was 0.47%. If seen from the figure Annual Malaria Incidence (AMI) that cases of clinical malaria for one year in an area per 1000 population in 2012 is 5.77% [8,9].

According to the epidemiological triangle concept (Trias Epidemiology) which gives an overview of the three main factors that play a role in the disease occurs are: host, agent, and environment. Host factors consist of all things human beings relating with incidence of a disease such as age, gender, occupation, heredity, race, body anatomy and nutritional status. Agent factor is a substance that can cause a disease. Environment factors are all external conditions and influences affecting the life and development of organisms such as the physical environment, biological, and social [10].

Harmendo research in 2008 in the Work Area Health Center District Sungailiat Kenanga Bangka regency, stated that the factors that influence the incidence of malaria namely the installation of netting on ventilation, the condition of the ceiling, out at night, and use the nets have the possibility of risk of malaria by 97 %. In addition, a study was conducted by Supri Ahmadi in 2008 in the District of Muara Enim, also stated that people who do not use mosquito nets at bedtime had 4.06 times the risk of malaria is greater than the people who use the nets. Pamela Ayu Aprilia research in 2009, stating that the risk factor affect the incidence of malaria is not put wire netting on home ventilation, the condition of the ceiling, the wall density, the presence of the bushes around the house, where the moat around the house, and the presence of cattle sheds around the house [11-13].

Strategies to control the epidemic of the disease depend on the surveillance to find new cases, followed by prompt action in controlling the epidemic. The discovery of new malaria cases should be supported by Geographic Information Systems (GIS) to describe and predict geographic patterns and time of disease transmission by vectors and disease prevalence. GIS can support spatial decision-making, in addition to providing an overview of a phenomenon, can provide information about the location, distribution of the phenomenon in the region, especially the increasing trend of malaria cases [14,15].

The use of GIS in public health can be used to describe the risk of malaria. GIS provide the right data to the visual and epidemiological data analysis, show trends, dependencies and relationships that may be difficult to be covered in a table format. In addition to the GIS can provide information about community health resources, specific diseases and other health events can be mapped in relation to the environmental and health effects of existing social infrastructure. This becomes very useful for mapping the risk of disease, identifying distribution patterns of disease, monitoring disease surveillance and response activities, evaluate the accessibility to health care facilities and the estimated range of disease outbreaks [14,16].

Based on the distribution, trends, and distribution of malaria, the formulation of the problem in this study was to determine the risk factors associated with the incidence of Malaria and spreading in Sijunjung district in 2013.

METHOD

This study is an analytical study using case control study design. The research was conducted in the District Sijunjung from January to July 2013 with 58 respondents and 58 cases of group control group respondents. Method of sampling in this study is to use simple random sampling method. Furthermore, the data were processed using univariate and bivariate analyzes using Mc Nemar ujistatistik with 95% confidence level (α = 0,05). Multivariate analysis used logistic regression.

RESULT

Sijunjung district is one of the districts in the province of West Sumatra, where Sijunjung district located in the eastern part of West Sumatra province. The total area of this district tends to ± 3130.4 km2 with boundaries. Geographically located at the earth's meridian, between 0º18'43"-1º41'46" south latitude, and 100º46'50-101º53'50" east

longitude. This district is generally the hilly topography with an altitude between 100-1500 masl (meters above sea level) with the average temperature ranges between 21-33 ºC. Sijunjung district area is 7.41% of the area of West Sumatra province is mostly forest (51.03%), consists of 8 (eight) District with 60 Nagari and 1 village,. The county is the largest districts Sijunjung Kamang New District with an area of 837.8 km^2 and ± district with the smallest area is the District Kupitan with area is ± 81.61 km^2 [9].

Table 1 Relationship bushes where the incidence of malaria in the district Sijunjung 2013

Case	Control						OR (95% CI)	P value
	There are bushes		No bushes		Total			
	f	%	f	%	f	%		
Bushes	10	58.82	20	48.78	30	51.72	2.857 (1.2081-6.75)	0.012
No bushes	7	41.18	21	51.22	28	48.22		
Total	17	100	41	100	65	100		

Relationship where the bushes with malaria incidence in Sijunjung district in 2013 can be seen in Table 1 that p value <0.05 (p = 0.012), meaning that there is a significant association between the presence of the bushes with malaria incidence. Also obtained 20 respondents (48.78%) were suffering from malaria have shrubs around the house and are not suffering from malaria do not have shrubs around the house. Then there are 7 respondents (41.18%) were suffering from malaria do not have shrubs around the house and are not suffering from malaria have shrubs around the house. Based on statistical calculations obtained OR = 2.857 (95% CI: 1.208 to 6.756) means that respondents who have bushes around the house 2.85 times the risk of contracting malaria compared with those who did not have the bushes around the house and the presence of shrubs a risk factor for the incidence of malaria in the district Sijunjung Year 2013.

Table 2 Relationship existence ditch / gutter with the incidence of malaria in the district Sijunjung 2013

Case	Control						OR (95% CI)	P value
	There are ditch		No ditch		Total			
	F	%	f	%	f	%		
Ditch	43	87.76	5	55.56	48	82.76	0.83 (0.254-2.730)	0.773
No ditch	6	12.24	4	44.44	10	17.24		
Total	49	100	9	100	58	100		

Table 2 shows there was no significant correlation between the presence of trenches/ditches with malaria incidence. Statistical calculations ditch, with OR = 0.833 (95% CI: 0.254 to 2.730) means the existence of the ditch / gutter is not a risk factor for the incidence of malaria. While Table 3 shows a significant association between the use of wire gauze on ventilation with malaria incidence (p = 0.000. Statistical calculations derived from OR = 6.750 (95 % CI : 2.631 to 19.290) means not wearing a wire netting is a risk factor for the incidence of malaria.

Table 3 Relations with the use of wire netting in malaria incidence Sijunjung district in 2013

Case	Control						OR (95% CI)	P value
	Don't use wire netting		Use wire netting		Total			
	f	%	F	%	F	%		
Wire netting	25	86.21	27	93.10	52	89.66	6.750 (2.361-19.29)	0.000
No wire	4	13.79	2	6.90	6	10.34		
Total	29	100	29	100	58	100		

Table 4 shows a significant association between the attic of the house with the incidence of malaria.

Table 4. Relationship ceiling with malaria incidence in the District Sijunjung 2013

Case	Control						OR (95% CI)	P value
	There is no ceiling		There is ceiling		Total			
	f	%	f	%	F	%		
No ceiling	0	0	25	49.02	25	43.10	3.571 (1.544-8.257)	0.001
Ceiling	7	100	26	50.98	33	56.90		
Total	7	100	51	100	58	100		

Relationship with the level of knowledge of the incidence of malaria in the district Sijunjung in 2013 can be seen in Table 5

Table 5 Relationship level knowledge with malaria incidence in the District Sijunjung 2013

Case	Control						OR (95% CI)	P value
	Low Knowledge		High Knowledge		Total			
	f	%	f	%	F	%		
Low Knowledge	3	42.68	14	27.45	17	29.31	3.500 (1.152-10.633)	0.019
High Knowledge	4	57.14	37	72.55	41	70.69		
Total	7	100	51	100	58	100		

Table 6 Relationship habits outside the house at night with the incidence of malaria in the district in 2013 Sijunjung

Case	Control						OR (95% CI)	P value
	Go outside at night		Don't go outside at night		Total			
	f	%	f	%	f	%		
Go outside at night	14	70	32	84.1	46	79.31		
Don't go outside at night	6	30	6	15.79	12	20.69	5.333 (2.23-12.755)	0.0000
Total	20	100	38	100	58	100		

The use of mosquito nets relationship Sijunjung district malaria incidence in the year 2013 can be seen in Table 7

Table 7 Relationship with the use of mosquito nets in malaria incidence Sijunjung district in 2013

Kasus	Kontrol						OR (95% CI)	P Value
	Don't use mosquito nets		Use mosquito nets		Total			
	f	%	f	%	f	%		
Don't use mosquito net	38	88.37	15	100	53	91.38		
Use mosquito net	5	11.63	0	0	5	8.62	3.000 (1.09-8.254)	0.026
Total	43	100	15	100	58	100		

Analysis results in Table 8, was found a significant independent variables likely to contribute to the occurrence of malaria, namely the existence of the house attic with p value 0.0065. Obtained from the calculation of the odds ratio test statistic = 5.2859 (95% CI: 1.592 to 17.367). That is, respondents who have no loft homes are at risk of malaria by 5.285 times compared with those who had a house attic, a significant relationship with p = 0.0065. Thus the dominant factor most at risk of malaria in the Sijunjung district in 2013, namely the attic house.

Table 8 Dominan Risk Factors Affecting Malaria Incidence

Variabele	OR	95 % CI	Coefficient	SE	P value
Bush	1.9771	0.7493 -5.2169	0.6816	0.4950	0.1685
Wire netting	3.5198	1.122 -11.0326	1.2584	0.5829	0.0309*
Attic house	5.2589	1.5922 - 17.3697	1.6599	0.6096	0.0065*
Knowledge	1.3690	0.4150 -4.5163	0.3141	0.6090	0.6060
Go outside at night	4.6477	1.7544-12.3125	1.5364	0.4971	0.0020*
Use netting	4.8032	1.2289-18.7743	1.5693	0.6955	0.0241*

DISCUSSION

The presence of shrubs (vegetation) may reduce sunlight lush enter/penetrate soil surface, so that the surrounding environment will be shady and moist. This condition is good place for rest for mosquitoes and mosquito breeding places also under the bush there is stagnant water [12]. The results of this study together with research conducted by Ayu Aprilia Pamela (2009) in the Village District Bener Ketosari Purwerejo District found that respondents who live in homes that are surrounding bushes are at risk for malaria disease 0.18 times greater than the families who live in homes that are not in the surrounding bushes OR 0.18 with 95 % CI : 0.04-0.8 [12].

In theory it is said that the presence of shrubs malaria is a risk factor, because it serves as a resting and breeding place, in line with this study, because the study researchers looked at breeding places of mosquitoes that are around the house respondents. Bush proven as one of the risk factors are also caused due to the proportion of exposure in the case group was greater in the control group.

Based on the value of OR can be said to those who did not wear a wire gauze on the ventilation of 6.75 times the risk of malaria compared with those who wear a wire gauze on the ventilation and the use of wire netting on ventilation are risk factors on the incidence of malaria in the district Sijunjung Year 2013. Observations and interviews with the researchers that many of the respondents in the District Sijunjung not put mosquito netting on his home ventilation, there is also a partially installed, and there are also installed but there is a hole. In the absence of mosquito netting on home ventilation, will facilitate the Anopheles mosquitoes into the house at night. This course will facilitate the contact between the occupants of the house with the mosquito-borne malaria, thereby increasing the risk of malaria transmission higher than the ventilated house mosquito netting attached.

The results of research conducted together with Hasan Husin (2007) In the Work Area Sukamerindu River District Health Center Serut Bengkulu found that respondents who did not wear a wire gauze on ventilation are at risk for malaria disease 3.71 times greater compared with those who wear a wire gauze on ventilation (OR = 3.71 with 95 % CI : 1.808 to 7.597) [18].

Statistical test results obtained p value <0.05 (p = 0.001), meaning that there is a significant association between the attic of the house with the incidence of malaria. Also obtained 25 respondents (49.02%) were suffering from malaria did not wear the attic of the house and do not suffer from malaria wearing attic. Then 7 respondents (100%) were suffering from malaria using the attic of the house and do not suffer from malaria did not wear the attic. Based on statistical calculations obtained OR = 3.571 (95% CI: 1.544 to 8.257) meaning no loft home is a risk factor for the incidence of malaria. Based on the value of OR can be said that there is no attic respondents had 3.57 times the risk of malaria compared with those who have the presence of the attic in the house and the attic is a risk factor on the incidence of malaria in the district Sijunjung Year 2013.

The results of this study together with research conducted by Harmendo (2008) in the Work Area Health Center District Sungailiat Kenanga Bangka found that respondents who do not have a home attic at risk for malaria 4.7 times greater than the respondents with a loft (OR = 4.7 with 95% CI: 2.38 to 9.37) [11]. This is due to the home office was not given the entire attic will facilitate the entry of mosquitoes into the house. Attic is a room divider wall with the top of a roof made of wood, bamboo wicker

internit smooth. If there is no attic means there is a hole or a gap between the walls with a roof so that mosquitoes are more freely into the house. Thus the risk of contact between the occupants of the house with Anopheles mosquitoes are larger than existing homes attic [12].

Relationship with the level of knowledge of the incidence of malaria in the district Sijunjung in 2013 can be seen in Table 5 obtained of p value < 0.05 ($p = 0.019$), meaning that there is a significant relationship between the level of knowledge of the incidence of malaria. Statistical calculations derived from OR = 3.5 (95 % CI : 1.152 to 10.633) means that the level of knowledge of risk factors on the incidence of malaria. OR can be said to be based on the value of the low knowledge respondents who do not have 3.5 times the risk of contracting malaria compared with respondents with high knowledge and knowledge level is a risk factor on the incidence of malaria in the district Sijunjung Year 2013.

Low knowledge causes individual or a family is difficult to identify the disease, the cause of the disease and efforts to prevent and eradicate malaria. As a result, the risk of getting the disease malaria is higher compared with individuals or families who have a good knowledge. Knowledge plays a very important role for the formation of a person's behavior, including behaviors in the prevention and eradication of malaria. Observations and interviews found that low levels of family knowledge can not be separated from the low levels that have been passed by the head of the family or a housewife as decision makers in the home tangga.Hasil this study is in line with research conducted by Efri Nurdin (2011) In Area Gold Mining District IV District Nagari Sijunjung found that respondents who had low knowledge are at risk for malaria are 22.52 times more likely than respondents who have a high knowledge (OR = 22.52 with 95%CI:4,89-103,59) [19].

Relationship habits outside the house at night with the incidence of malaria in the district Sijunjung in 2013 can be seen in Table 6 obtained $p = 0.000$ meaning that there is a significant relationship habits out of the house at night with the incidence of malaria. Obtained from statistical calculations obtained OR = 5.333 (95 % CI: 2.230 to 12.755) means habit out of the house at night is a risk factor on the incidence of malaria. OR can be said to be based on the value of the respondents who have a habit of going out at night had 5.3 times the risk of contracting malaria compared with those who do not have the habit of going out at night and a day habit out of the house at night is a risk factor for the incidence of malaria in Sijunjung district in 2013.

The results of this study together with the study of Javan Samuel Franklyn (2006) In the Work Area Health Center Bosnik East Biak Biak Papua Noemfoor East found that respondents who have a habit of being outdoors at night 4.68 times the risk of malaria than those who do not have the habit out of the house at night (OR = 4.68 with 95% CI 1.29 to 16.983) [20].

This suggests that the habit outdoors at night at risk of contact between healthy people with mosquito Anopheles spp. which requires blood to meet gonotropik cycle. If mosquitoes are biting containing sporozoid in saliva, then the chances of contracting malaria will be even greater. Based on the location of the interview study found that activity in the evening outside the house chatting, staying up fishing and defecate in the back of the house [20].

Table 7 shows a significant correlation with the incidence of malaria bed net usage. Also obtained 15 respondents (100 %) were suffering from malaria nets and are

not suffering from malaria nets. Statistical calculations derived from OR = 3.000 (95 % CI: 1.660 to 19.336) means that no nets were risk factors for the incidence of malaria. OR can be said to be based on the value of the respondents who did not use bed nets have 3 times the risk of contracting malaria compared with respondents who use mosquito nets and mosquito net use is a risk factor on the incidence of malaria in the district Sijunjung Year 2013.The results are consistent with research Yudi Pradipta (2012) In the city of Padang found that respondents who did not use mosquito nets 4.48 times the risk of contracting malaria nets dibandigkan respondents (OR = 4.48 with 95 % CI: 1.339 to 14.991) [21].

The use of mosquito nets is the most effective efforts to prevent mosquitoes while sleeping. The use of mosquito nets is better than drug use mosquito repellent. Among these is to avoid the entry of insecticides in the body through inhalation or skin tissue. The use of mosquito nets while sleeping gives comfort during sleep because mosquitoes are not disturbed by noise during flight and avoid the risk of mosquito bites either as vectors of malaria or not. Results of interviews obtained information that the respondents did not use mosquito nets among others, due to the distribution of insecticide-treated bed nets (net impregnated by the preferred health center to a house that had a toddler, was hot and sultry . besides that though there is a mosquito net at their home but the condition and how to set it up is not good and the opportunity for the entry of mosquitoes.

CONCLUSION

Objectives and results of research on spatial analysis of risk factors for the incidence of malaria in the district Sijunjung in 2013 , it can be concluded as follows Relationship environmental risk factors and individual risk factors on the incidence of malaria in the district in 2013 Sijunjung environmental risk factors that have a significant relationship is the presence of shrubs bushes , use of wire netting on ventilation , the attic of the house while the individual risk factors is the level of knowledge , habits out of the house at night , the habit of using bed nets . The results that the existence of the trench / ditch there was no correlation with the incidence of malaria . The most dominant risk factors affecting the incidence of malaria in the district in 2012 was the presence Sijunjung attic , It is known that the spatial distribution of malaria incidence in Sijunjung district there are several sub- groups are spreading and some districts only a few cases . The distribution of malaria cases based on environmental conditions found in many cases living in the bushes near an anopheles mosquito breeding sites

REFERENCES

1. Ministry of Health Indonesia. Guidelines for Case Management of Malaria in Indonesia. Directorate General of Disease Control & Environmental Health. Jakarta: Department of Health Indoneia; 2008.
2. World Health Organization.World Malaria Report 2011 [Online]. Dalam: www.who.int/who_malaria [Sabtu,8 Desember 2012].
3. Ministry of Health Indonesia. Guidelines for Case Management of Malaria in Indonesia. Directorate General of Disease Control & Environmental Health. Jakarta: Department of Health Indoneia; 2011.

4. Friaraiyatini, Soedjajadi K., Y. Ririh Environment and Behavior Influence People Against Malaria incidence in the district. South Barito Central Kalimantan. Surabaya: Airlangga University. Vol. 2, No.. 2, January 2006:121 - 128.
5. Ministry of Health. Health Data Profile 2012.Kementerian Health of the Republic of Indonesia of Indonesia. Jakarta: Department of Health; 2012.
6. West Sumatra Provincial Health Office. West Sumatra Health Profile 2010. Padang: West Sumatra Provincial Health Office; 2010.
7. Data Health Department/District Health Information. Mentawai Islands of West Sumatra Province. Padang: Department of Health; 2011.
8. Sijunjung District Health Office. Sijunjung District Health Profile of the Year 2011
9. Sijunjung District Health Office . Sijunjung District Health Profile 2012. Sijunjung: DHO Sijunjung; 2012.
10. Bustan, M.N. Introduction to Epidemiology Revised Edition. Jakarta: Rineka Copyright; 2006.
11. Harmendo. Malaria Risk in the Work Area Genesis Health Center District Sungailiat Kenanga Bangka [thesis]. Semarang. Graduate Program in Environmental Health, University of Diponegoro in Semarang; 2008.
12. Pamela, Aprilia. Hubungan Physical Environment Around the Home And Home With Malaria incidence in the Village District Bener Ketosari Purworejo.Surakarta District. Health Studies Program Faculty of Health Sciences, University of Muhammadiyah Surakarta; 2009.
13. Ahmadi, Supri. Malaria incidence Risk Factors In the village of Tanjung Lubuk Nipis District Court District of Muara Enim [thesis]. Graduate Program in Environmental Health, University of Diponegoro in Semarang; 2008.
14. Use of Geographic Information Systems (GIS) in the Control of Malaria in Mamuju [On line]. In www.muslimpinang.wordpress.com [January 3, 2013]
15. Presentation of Spatial Data [On Line] In http://www.dephut.go.id/ [January 16, 2011]
16. Malaria epidemiology in GIS [Online] In www.muslimpinang.wordpress.com [January 3, 2013]
17. Nurbayanti, Lara. Malaria incidence Risk Factors In Health Center Mayong I Jepara regency. Semarang: Diponegoro University. Vol 2, No. 2, January 2013
18. Husin, Hasan. Analysis of Risk Factors in Operational Area malaria incidence Sukamerindu River District Health Center Serut Bengkulu [thesis]. Graduate Program in Environmental Health, University of Diponegoro in Semarang; 2007.
19. Nurdin, Efri. Factors-Factors Associated With Malaria incidence in Region IV Gold Mining District Nagari Sijunjung district in 2011. Science Program of the Faculty of Public Health Medicine, University of Andalas; 2011.
20. Javan, Semul Franklyn. Analysis of Risk Factors in Operational Area malaria incidence Bosnik Health Center East Biak Biak Papua Noemfoor East [thesis]. Graduate Program in Environmental Health, University of Diponegoro in Semarang; 2006
21. Pradipta Yudi Malaria incidence Risk Factors With Spatial Approach In Padang City in 2012 Andalas University School of Public Health 2012.

CORRELATION BETWEEN PHYSICAL ENVIRONMENT OF HOUSE, NEIGHBORHOOD, CULTURAL AND SOCIAL ENVIRONMENT WITH MALARIA OCCURRENCE
(Study in Sepaku Health Center, Penajam Paser Utara district)

Siswanto, Risva, Mas Ratu
Public Health Faculty, Mulawarman University
Correspondence:

ABSTRACT

Background: In Indonesia, 60% of approximately 210 million (more than 100 million) people live in endemic areas and have risk of contracting malaria. In Sepaku Health Center there was as many as 994 cases of malaria in 2012.
Method: This was a case control study. Sample was 182 respondents, consisted of 91 cases and 91 controls (ratio 1:1). Analysis used Chi Square and Odd Ratio.
Result: The result showed malaria significantly related to physical environment ($p<0.0001$, OR=10.059), neighborhood environment ($p<0.0001$,OR=12267), and socio-cultural environment ($p=0.002$, OR=2.685).
Conclusion: Sepaku Health Center must pay attention to physical and socio-cultural environment that related to malaria occurance.
Keywords: environment, malaria.

BACKGROUND

Malaria is an infectious disease caused by Plasmodium through Anopheles bite. There are four species of Plasmodium that cause malaria in human, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* [1]. Indonesia is one of high risk countries of malaria. In 2007, Indonesia had 396 endemic areas out of 495 districts. Around 45 % population live in endemic areas and have risk of malaria infection. Number of malaria cases in 2006 was 2 million, which decreased in 2007 to 1.7 million [2]. Spreading of malaria is influenced by host (human), agent (Plasmodium) and environment [1]. The environment factors that influence malaria consisted of biologic, physical (temperature, humidity, rainfall, height, wind), and socio-cultural environment. Epidemiological data on those environments are needed to overcome malaria problem.

In East Kalimantan malaria progressively extended and flatted. Data from Health Office showed malaria have distriuted to all areas or 14 districts/cities. The highest malaria cases based on Annual Parasite Incidence (API) was Penajam Paser Utara (PPU) district. In 2010, there was 1.368 positive patients out of 2.063 examination. In 2012, malaria spread to 11 health centers in 4 districts, with 705 positive cases out of 1.644 examination. Most cases was in Sepaku Health Center (297 cases), followed by Sotek Healtlh Center (180 cases) and Maridan Health Center (110 cases).

METHOD

This was an analytic observational study with case control design, conducted in Sepaku 1 Health Center, PPU, on May 2013. Population was all clinical malaria patients in Sepaku 1 Health Center on Januray-December 2012. Sample consisted of 91 cases (malaria patients) and 91 controls (not malaria patients). Inclusion criteria for respondents of both group was willingness to engage with this study and have been living in Sepaku at least one year in a row. As to control group, respondents must live in same area with case, and distance from ricefield less than 2 km. Sample was taken using simple random sampling. Independent variable in this research was physical environment of house, neighborhood environment, and social culture environment. Dependent variable was malaria occurence.

Collection of data used questionnaire on physical environment of house (hygiene, wall type, hole at wall, wall color, ventilation, wire netting, existence of ceiling, garbage, housewares), neighborhood environment (existence of rice field, brushwood, garden, forest, water pond, ditch, moat, fish pond, sewerage), and sociocultural environment (habit to use mosquito net, mosquito coils, mosquito spray, repellent, habit of going out or being outdoors at night). Physical environmental was categorized as clean and not clean, neighborhood environment was categorized as healthy and unhealty, while sociocultural environments was categorized as supported and unsupported. Validity and realibility test of questionnaire was done prior to study.

RESULT

Our result showed proportion of malaria was higher in male than women (Table 1). Most cases was in age group of 18-25, while least cases was found in elderly (66-73 years). Table 2 showed there were significant relationship between physical environmen ($p<0,0001$), neighborhood environment ($p<0.0001$) and sociocultural environment ($p=0.002$) with malaria in Sepaku 1 Health Center.

Table 1. Distribution of malaria occurence based on responden characteristics

Variables	Occurance of Malaria Disease				Σ	%
	Case	%	Control	%		
Age Classification						
10 – 17	7	7.7	2	2.2	9	4.9
18– 25	33	36.3	35	38.5	68	37.4
26 – 33	19	20.9	21	23.1	40	22
34 – 41	8	8.8	16	17.6	24	13.2
42 – 49	8	8.8	5	5.5	13	7.1
50 – 57	7	7.7	8	8.8	15	8.2
58 – 65	7	7.7	3	3.3	10	5.5
66 – 73	2	2.2	1	1.1	3	1.6
Gender						
Female	25	27.5	36	39.6	61	33.5
Male	66	72.5	55	60.4	121	66.5

Table 2. Relation between physical environment, neighborhood environment, sociocultural environment and malaria

Item		Occurance of Malaria Disease				Σ	%	P value	OR 95% Cl
		Case	%	Control	%				
Physical Environmental House	Risk	78	85,7	34	37,4	122	61.5	0,000	10,059
	Unrisk	13	14,3	57	62,6	70	38.5		
Around of Environmental House	Risk	84	92,3	45	49,5	129	70,9	0,000	12,267
	Unrisk	7	7,7	46	50,5	53	29		
Social Culture Environmental	Risk	58	63,7	36	39,6	94	51,6	0,002	2,685
	Unrisk	33	36,3	55	60,4	88	48,4		
		91	100	91	100	182	100		

DISCUSSION

Relation between physical environmental and occurence of malaria disease

Respondent with unhealthy physical environment had 10 times risk to get malaria. According to our observation result, one of factors that contributed was open ventilation. our result showed respondents living in house without wire netting on ventilation was 74.7 %. Type of ventilation in most respondents did not suit netting. Opened ventilation may facilitate mosquitoes enter into house, and this was similar to Husain (2007) [3].

Other factor was wall condition. Most respondents had embrasure wooden wall, so that mosquitoes can easily pass into the house. This result as according to result of research of Husain (2007) [3] also find that patient of malaria mostly lived in house with improper wall. According to Mukono [4] house contruction with wall that is not tightly closed enabling malaria infection. Besides wall, ceiling also plays important role to avoid mosquitoes entering house. A study conducted by Widaryani (2006) showed malaria related to absent of ceiling. Likewise, Franklyn (2007) also proved lack of ceiling as a risk factor of malaria.

Third factor found in unhealthy house that owned too many housewares, and not regularly cleaned. The housewares were mostly not well maintened, and served as mosquitoes resting place. Fourth factor was less indoor lighting, because respondents seldom open their window during morning and daytime. Moroover, housewares also hindered to come into house. This condition of course can cause situation of humid and dark house, so that can become resting and propagation place of mosquito. According to Barodji, place take a rest mosquito of anopheles in general in place having high humidity and low light intensity. Besides, Harijanto revealed that humidity and temperature influence propagation of mosquito parasite [5]. According to Sudarman, Anopheles mosquitoes rest on wall, clothes, gauze, housewares, cupboard, and ceiling.

Based on this study, we concluded that most respondent did not meet standar of healthy house according to regulation of Ministry of Health (*Permenkes*) number 829 (1999).

Relation between neighborhood environment and occurence of malaria

Our result showed unhealthy neighborhood environment increase risk 12 times higher to contract with malaria. In in case, 92.3 % respondents of our study lived in neighborhood environment that was not meet health requirement, consisted of rice field (55 respondents), plantation (59), forest (78), bushwood (87), big grove/moat (79), ditch (82), pool (56), and sewerage (77) as places for malaria vector propagation. This result similar to Parewasi in Makmur (2002), who also showed that malaria patient lived in coastal, marshy areas which surrounded by neighborhood environment such as forest and rice field.

Our results showed only 7.7 % case group lived in a healthy neighborhood environment. Most of respondents lived near rice field, forest in and pool. Rice fields usually serve as place for malaria vector propagation. According to MoH (2006), rice field is one of breeding places along with swamp, wellspring, pool, river estuary, brackish water pond, rainwater pond and river-based irrigation. Other factor, forests, may increase humidity. Harijanto stated humidity and temperature influence propagation of parasite in mosquitoes [5]. Pools also important as one of the breeding place of mosquito larva. Therefore, MoH suggested incorporating fishes into pool as predator to larvae.

The effort to keep environmental clean is important on ecosystem balancing in order to increase health status, prevent from disease, thus decreasing incidence. Lack of paying attention hygiene of environment about hand in glove residence of its bearing with willingness at society to do so because lack of awareness of society for the importance of hygiene of environment besides workdload of society like going to cleaner which confiscating many their time which cause society less is paying attention of hygiene of environment. This matter as according to research of Prabowo (2004) where lessening breeding place of mosquito with activity of PSN around residence environment, killing to snap fingers and adult mosquito and also hygiene of environment represent one of the preventive effort in avoiding mosquito bite of malaria [7].

According to Fathi (2005), environmental interaction with disease development have a vice versa effect. Therefore, do not him of this condition of environment around settlement of responder very having an effect on to occurence of existing malaria. The suggest of keep cleaning society will be environmental around by conducting sweeping to place - resort and breeding place of mosquito of anopheles like routine clean moat or ditch, cleaning bush, looking after fish eater of larva, and plant or look after plant which do not in taking a fancy to mosquito like Akar Wangi, Serai Wangi, Tapak Dara, Lavender, and orange tree. Besides can dissipate this crop mosquito also earn environmental estetis of house.

Relation between social culture environmental and occurence of malaria

Respondents with unsupported sociocultural environment had 2.6 times risk in contracting malaria compare with the supported ones. One of dominant sociocultural factors that had effect on malaria occurence was habit of beeing outdoors at night. Night time activity mostly done men, and related to their occupation. Bionomic of vector in Sepaku was exophilic and exophagic. Therefore, outdoor activities during night increased risk of mosquito's bite. Darmadi (2002) prove that outdoor activity between 09.00 to 10.00 pm (during mosquito's peak activity) was closely related to malaria

occurence. Anopheles also tends to have exophagic character. Thereore, people with outdoors activities at night had greater risk to expose with mosquito bite. Yawan (2006) showed being outdoor at night increase risk 4,68 times to get mosquito bite that will lead to malaria.

Using bed net while sleeping at night may reduce risk of malaria, because Anopheles are mostly nocturnal that bite during night. Pranoto stated feeding habit of Anopheles mosquito varry depends on species, some bite from dusk until before midnight, some can bite all night long, some start to bite at 06.30 to 10.00 pm [8]. Bed net is one of ways to prevent mosquito bite. According to of Sulistiyo (2006), bed net related to malaria occurence. Besides bed net, anti-mosquito was also considered as useful to avoid malaria. Prabowo (2004) revealed one of actions to avoid mosquito bite of vital importance was use anti mosquito/repellent in form of repellent cream, spray, coils during night time prevent mosquito bite [7]. Husain (2007) also proved always using repellent cream when outdoor may prevent from malaria [3].

Other factor in Sepaku that may cause malaria was hanging clothes habit. Even in control group, 60.4 % respondents had the habit. Selly stated Anopheles likes dark places to rest after feeding, and usually choose hanging clothes. Franklin (2006) showed family with hanging clothes habit have higher risk of malaria occurence (OR=16.923).

Limitation of Research

This research, there are some natural resistance or limitation by researcher is weather factor and residence factor. This research is conducted by at the rains so that researcher mobility pursued by when doing research like slippery and muddy road. Besides natural resistance by researcher is residence, this matter because in research area do not there are or lodging of stay for researcher to rest. So that researcher perforced to look for - citizen house searching which will in making place rest.

CONCLUSION

There are significant relationship between physical environment, neighborhood environment and sociocultural environment with malaria occurence in Sepaku, Penajam Paser Utara. Population is expected to pay attention, improve, and repair their environment.

Based on this study, we expected to society in region work Puskesmas Sepaku 1 Sub-Province of Penajam Paser Utara to be can pay attention and improve; repair the condition of house environment like wiring gauze at house ventilation, improve; repairing resident house desain by changing wall type which there are hole or have is weak (jabuk) with ligneous strong and new board type of Kayu Ulin with installation which is meeting or change wall type with stronger materials like is made from cement, arranging to arrange situation of furniture regularly in order not to hinder sunlight which enter into housewares keep cleaning and house, using mosquito net and drug anti mosquito when bedtime between two lights, cleaning environment around of house by conducting sweeping to moat or ditch routinely hygiene goodness and also his current fluency. Besides Society can plant plant around which do not in taking a fancy to mosquito like Akar Wangi, Serai Wangi, Tapak Dara, Lavender, and orange tree. Killing wiggler by looking after eater fish snap fingers at (tin head, gupi, mujair) at

pool in around of house, rice field, swamp and and fish found which do not be looked after.

REFERENCES

1. Bruce-Chwatt, L.J. Essential Malariology. 2nd edition. London: William Heinemann Medical Books Ltd.; 1985.
2. Keputusan Minister for Public Health of RI No. 293/MENKES/SK/IV/2009. 28 April 2009 About Eliminasi Malaria of di Indonesia. Director General of P2Pl. Depkes.
3. Husin, Amirsyah. 2002. Analysis Supplementary Factor Height of Insidens Malaria in Gugus Kepulauan Aceh. Jurnal Doctor of YARSI. Jakarta.
4. Mukono HJ. (1999). Principle of Environmental Base of Surabaya : Airlangga University Press.
5. Harijanto, P.N. 2000. Malaria, Epidemiology, Patogenesis, Manifestasi Klinis and Handling. Jakarta: EGC.
6. Erdinaldkk. 2006. Factor - Factor Related To Occurence Of Malaria in District Of Middle Left Camphor, Sub-Province Camphor, 2005 / 2006. Jurnal Kesehatan. Jakarta : Departemen Kesehatan Lingkungan, FKM UI 2006.
7. Prabowo, A. 2004. Malaria, Preventing and Overcoming. Jakarta: Puspaswara.
8. Pranoto. 1980. Some Behavioral Aspect of An. Farauti in Klademak II A, Shove. Mirror of Dunia Kedokteran. Jakarta.

THE EFFECT OF *Annona muricata* EXTRACT TO *Aedes aegypti* LARVAE DEATH

Dwi Sutiningsih
Public Health Faculty Diponegoro University Semarang
Correspondence: dwisningsih@yahoo.co.id

ABSTRACT

Background: The control of DHF vector is still emphasized on the usage of chemical insecticides, which repeatedly can cause vector resistance, death of other animals which is non target and pollution. Therefore, it must be looked for other way to control DHF vector that is by using natural insecticide, one of them is by using plant. This research aims to do a test whether A. muricata extract can cause death of *Anopheles aconitus* larvae.
Method: This research method is using experimental plan with "*Post test only group design*"The result shows that there are death of *Ae. aegypti* larva after giving of A. muricata extract**.**
Results: The lowest concentration (0,01%) cause death of *Ae. aegypti* larva 8,35% and the highest concentration (0,4%) all *Ae. aegypti* larva are dead. Result of Anava test expressed that there are differences of the averase of larva death at various extract concentrations level of soursop root extract (*A. muricata L.*). The LSD *(Least Significant Different)* test indicate that not all couple average of value death of *Ae. aegypti* larvae are different significantly.
Conclusion: This study conclude that is the soursop root extract (*A. muricata L.*) can kill *Ae. aegypti* larva. The concentration that is able to kill *Ae. aegypti* larva at (LC50) is 0,15% and (LC90) is 0,27%. There is a significant difference of the averase of larva's *Ae. aegypti* death at various extract concentration of soursop root extract (*A. muricata L.*).
Keyword: Ae.aegypti, soursop(Annona muricata) root extract, Lethal Concentration 50 (LC_{50}) , Lethal Concentration 90(LC_{90})

BACKGROUND

Various kinds of insecticides have been used in vector control efforts as effective, its applicationis relatively easy and the results are known quickly. In addition to chemical insecticides is relatively expensive when used repeatedly can lead to resistance vector, the death of other non-target animals, and environmental pollution. It is therefore necessary to find another way to control the vector of DHF is by using biological insecticides [1]

Soursop plants have been known to contain active compounds which annonain, tannins, alkaloids which can be used as a biological insecticide.[2] Parts of plants that contain these compounds are still unripe fruit, seeds, leaves and roots.

According Purwohusodo (1997), soursop leaves and seeds that produce distilled liquid toxic levels of 10%. Liquid soursop leaves with lethal concentrations of 5.50% to 50% of the third instar larvae of Aedes and Culex mosquitoes within 48 hours. At a

concentration of 6.48% to 50% lethal larvae within 24 hours. While liquid soursop seeds with a concentration of 6.50% to 50% lethal larvae of Ae. aegypti within 48 hours[3]

Rislansyah research results (2000) showed that the soursop leaf extract at a concentration of 0.026% effective in killing larvae of Ae. aegypti by 50% and the 0.077% concentration to kill larvae of Ae. aegypti by 90% [6] While the results of Ruth research (2004) showed that the crude extract of soursop fruit with 0.20% concentration to kill larvae of Ae. aegypti by 50% and by 0.27% concentration to kill larvae of Ae. aegypti by 90%. This study aims to know soursop extract (A. muricata) can cause death Ae. Aegypti larvae by determining the value of Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90}).

METHOD

The population used in this study was Ae. aegypti larvae at the BPVRP Laboratory, Salatiga. While the study sample was the larvae of Ae. aegypti third larval instar with considerations on the mosquito organs of the body is already a fully-formed and relatively stable against environmental influences. The number of larvae per treatment as many as 20 larvae, the number of repeat 3 times. The sampling technique was done by random sampling.

***Preparation of A.muricata extract*.**

Preparation of A.muricata extract was using by percolation method with 70% ethanol. soursop roots extracted by soaking 10 simplicia part and then put in a closed vessel for at least 3 hours. Then the mob moved little by little into the percolator while each time pressed carefully. Determine of A. muricata extract concentration was using a stock solution concentration of 0.4% by mixing 1 ml of 70% ethanol with 100 ml of distilled water. Then a solution of diluted material taken in accordance with the required concentration [5].

Bioassay test

Larvae of Ae. Aegypti as much as 20 tail were included in each soursop root extract concentration (0.01%, 0.03%, 0.05%, 0.07%, 0.09%, 0.1%, 0.2%, 0.3% and 0.4%, 1 control) with repeat 3 times. pH of the media, media temperature and humidity at the beginning and end of the experiment recorded, and observed after 24 hours. After 24 hours, the number of deaths larvae of Ae. aegypti recorded and calculated at each concentration. If the number of larvae in the control group mortality of less than 5% was ignored, but if more than 20% should be repeated. If larval mortality in the control group between 5-20%, then to calculate the percent mortality of larvae at each concentration correction by using Abbott formula [7].

The results of the calculation of the death of the larvae of Ae. aegypti were analyzed using probit analysis to determine the LC_{50} and LC_{90} values. To determine the difference in death rate followed by ANOVA test with a confidence level of 0.05 . If you show any significant difference then followed by least significant defference test (LSD).

RESULTS

Environmental conditions such as pH, humidity and temperature can affect larval life. pH of the media in this study ranged from pH 6.5 to 7.2, while the medium temperature range from 26.5 ºC to 27ºC, humidity range 70-75%. In this case the pH of the media is still in the range of 6-8 suitable for larval development which is 6.3 to 7.2, as well as the temperature and humidity of the media is still in the range of optimum temperature suitable for larval development at 25-27ºC and humidity range of 60-80% or moisture optimum for larval development. So the magnitude of the pH of the media, media temperature and humidity when the research does not interfere with the development of larvae of *Ae. aegypti*. It can be concluded that the death of the larvae of *Ae. aegypti* is not caused by environmental factors such as pH of the media, media temperature and humidity [4].

The results showed that the lowest concentration of root extract of soursop cause mortality of 0.01% larval Ae. aegypti by 5%, while the highest concentration of 0.17% caused the death of the larvae of Ae. aegypti by 85%. The percentage mortality of larvae of Ae. aegypti after administration of root extract of soursop (A. muricata) during the 24-hour observation in the preliminary test are presented in Figure 1.

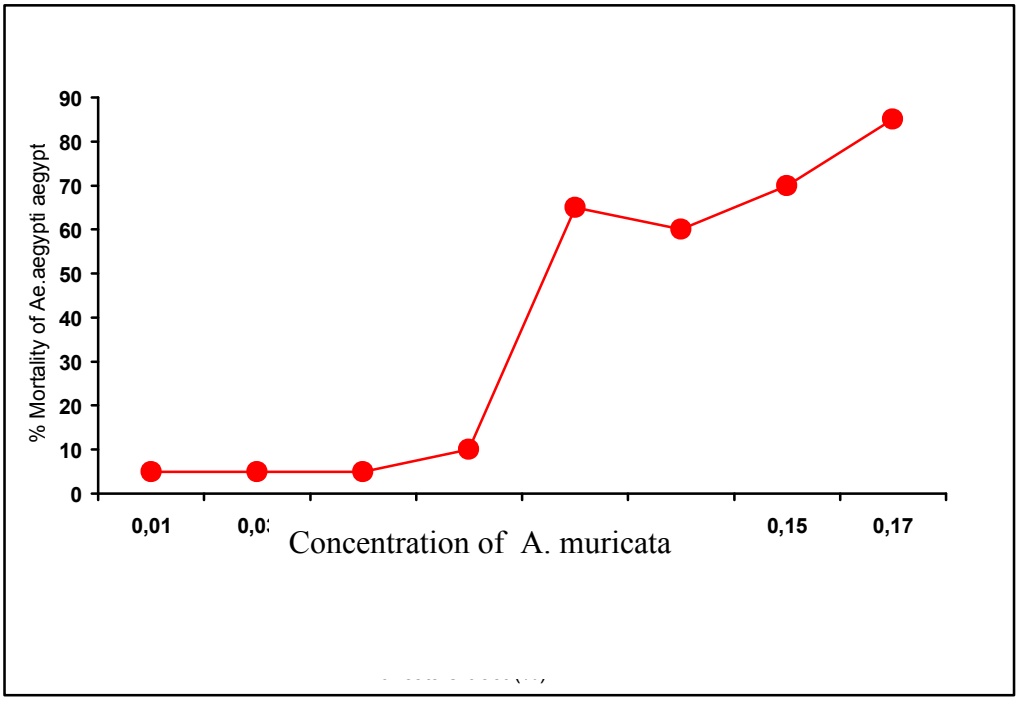

Figure 1. Mortality of*Ae. aegypti*larvae after *Annona muricata* extract at 24 hours.

Probit analysis results showed that the root extract of soursop LC50 value is of 0.15% and soursop root extract is LC90 of 0.27% means to kill larvae Ae.aegypti much as 90% within 24 hours it takes root extract concentration of 0.27 soursop %. Results of probit analysis in full can be seen in Table 1.

Table 1. Values of*Lethal Concentration* 50% (LC_{50}) and 90% (LC_{90}) of *Ae. aegypti*larvae after A*nnona muricata extract at* 24 hours.

Mortality (%)	A. muricataextract (%)	CI	Range (%)
50	0,15	0,95	0,12<LC< 0,17
90	0,27	0,95	0,23<LC< 0,33

DISCUSSION

The death of *Ae. aegypti* larvae probably caused by the active compounds from the roots of soursop (*A. muricata*) is annona in compounds, tannins, alkaloid. Annonain compound is the active ingredient that effective as a biological insecticide because it works as a contact insecticide that entry into the body through insect eksoskelet the intermediate tarsus at intermission. In addition, the compounds may also annonain as a stomach poison by going into the insect body through the mouth which resulted in the death of the larvae. Alkaloids have the same structure as the saponins that have properties such as saponins which can lower the surface tension of mucosal lining of the digestive tract so that the larval digestive tract wall become corrosive.Results of analysis of variance (ANOVA) there are differences in the average number of deaths larvae of Ae. aegypti at different levels of concentration of root extract of soursop (Annona muricata) is significantly ($p < 0,05$) [5].

Based on the results of LSD test for *A. muricata* concentration of 0.01%, 0.03%, 0.05%, 0.07%, 0.09%, 0.1%, 0.2% and 0.4% concentrations showed a concentration pairs significantly different to the death of the larvae of *Ae. aegypti*. This means that any concentration of root extract of soursop (*A. muricata*) has tested the power to kill or different toxic effects on the larvae of *Ae. aegypti*. In addition it might be due to the influence of the larvae themselves because at the time of sampling, namely the possibility of the third instar larvae age of the third instar larvae are not the same and is approaching the fourth instar. Possible toxic effects of late third instar larvae are more resistant to chemicals than the early third instar larvae.

This research, observation for 24 hours it is based on the criteria of efficacy, the mortality rates should reach at least 90 % within 24 hours. The results of this test indicate that the concentration of 0.3 % to be effective as a biological insecticide because the concentration is the concentration lethal to larvae 93.35 % in 24 hours.

According to the research Rislansyah (2000) of soursop leaf extract on larval Ae . aegypti obtained LC50 and LC90 of 0.027 % at 0.077 % .While the crude extract of soursop fruit by Ruth (2004) obtained LC50 was 0.20% , LC90 was 0.27%. At the root of soursop obtained LC50 of 0.15 % and 0.27% at the LC90 [6].

It can be concluded that the soursop leaf extract is more effective because it is used as a biological insecticide concentration of extract required less compared to other parts of the plant that is the raw fruit soursop and roots .This may be due to the active compound of soursop leaves is higher than fruits and roots.

CONCLUSION

Annona muricata extract can kill the larvae of *Ae. aegypti*, the lowest concentration of 0.01 % caused the death of the larvae of *Ae. aegypti* at 8.35 % and 0.4 % at the highest concentration caused of all the dead larvae (100 %), with LC50 and LC90 values of 0.15 % and 0.27 % LC90. There are differences in the average number of deaths larvae of *Ae. aegypti* at different levels of concentration of root extract of soursop (*A. muricata*) was significantly ($p< 0,05$).

REFERENCES

1. Kardinan, A. *Pestisida Nabati, Ramuan dan Aplikasi*. Penebar Swadaya, Jakarta, 2002.
2. Bambang, M. *Ramuan Tradisional untuk Penyakit DHF*. Penebar Swadaya, Jakarta, 2000.
3. Rukmana, R. *Usaha Tani Sirsak. Kanisius.* Yogyakarta, 2001.
4. Lounderhausen M, Leicht. W, Lieb F, Moeschler H dan Weiss H. *Mode Aksi Molekuler Annonain*. Ilmu Pengetahuan Pestisida.1991.
5. Robinson, T. *Kandungan Organik Tumbuhan Tingkat Tinggi*. ITB, Badung, 2003.
6. Rislansyah. *Daya Bunuh Ekstrak Daun Sirsak (Annona muricata Linn) terhadap Jentik Anopheles aconitus (Studi Eksperimen Laboratorium).* FKM UNDIP, Semarang, 2000. (Tidak dipublikasikan)
7. Komisi Pestisida. *Metode Standar Pengujian Efikasi Pestisida*. Departemen Pertanian, Jakarta, 1995.

COMMUNITY EMPOWERMENT TO REDUCE MALARIA DISESASE: AN ACTION RESEARCH IN BANJARNEGARA DISTRICT, CENTRAL JAVA

Bagoes Widjanarko[1], Suhartono[1], Mateus Sakundarno Adi[1], Kusnari[2]
[1]Faculty of Public Health Diponegoro University
[2]District Health Office of Banjarnegara
Correspondence: bagoes62@yahoo.com

ABSTRACT

Background: Malaria is preventable water related diseases caused by Plasmodium parasite and transmitted from people to people through infected mosquito bite. Since 1995, in Banjarnegara district, the malaria prevalence has been increasing. The ongoing program was mass fever survey to find malaria cases, mass treatment and vector control, however malaria prevalence still increasing from time to time. By examining the dynamic of transmission, the most effective way to prevent infection is by controlling risk factors. Unfortunately, people who live in endemic place are usually unaware.
Method: The action research was done during the year of 2000 to 2001 to empowered community in engaging social change to reduce malaria risk factors. Participatory activities were applied to facilitate community members to understand malaria disease.
Results: In the beginning malaria was commonly understood caused by bad food consumption. After serial participative activities such as socio drama, brain storming and village transect, community understands the cause, mode of transmission and how to prevent malaria. Commitment to work together was made to free their village from malaria disease. Breeding and resting places of malaria vector were identified. Social mobilization was introduced to practicing preventive action such as cultivated *ikan kepala timah* in their ponds, collecting money to purchase bed net and modified their activities to avoid from mosquito bite. *Jenang salak* was made as local product to enhance their economic capability. After a couple years, malaria prevalence was continuing decrease.
Conclusion: Empowered community members have adequate capacity to identify local health problem, using local resources, and manage problem solving activities.
Keywords: community empowerment, malaria

BACKGROUND

Malaria, water related infectious disease is still a part of major diseases in developing country like Indonesia. It is preventable and be cured, but still become a major cause of death in some countries. Malaria outbreak is frequently happen in many places in Indonesia including Central Java Province. Based on Mass Survey, malaria cases in Indonesia were nearly 1.5 million and 30 thousands of them die.

Malaria prevalence in Central Java was increasing in the year of 1995 to 1999. In Banjarnegara, the small city in Central Java Province, the prevalence was 1.59/1000, 2,72/1000 in the year of 1998, and become 5,46/1000. Mass fever survey, mass malaria cases treatment, community health education and vector control have been done to

prevent, diagnosed and cured malaria patients, so far the prevalence was still increasing (Banjarnegara District Report, 2000).

Malaria risk factors study in Banjarnegara got results that the presence of water ponds in surounding house, custom activities outside the home at night, construction of house building and lack of knowledge proven has relationship with malaria infection (Suhartono, 2001). To overcome these problems need to be planned a risk factor and prevention efforts involving individuals, families and communities through community empowerment (Labonte, 2008; Glanz, 2002). Empowered community would have new knowledge and capacity to identify their problem, raise alternative solution and make commitment to solve problem using local resources.

METHOD

In the preparation of community empowerment activity we developed networks involving local government, District Development Planning Board, Department of Education, Religious Sector, Department of Agriculture and, and Family Welfare Education. Through this network, it is expected to sustain prevention activities. A series of activities needs to be undertaken to provide for community leaders skill to become facilitators in mobilizing community participation (Labonte, 2008).

Method used is the Participatory Rural Appraisal. With this method are actively involved community members to take a role in recognizing the problem, choose and prioritize alternative solutions, and mobilize community participation to implement the commitment by utilizing local resources. Some of necessary equipment and materials were provided by the research team, just as a driver to motivate people to participate in providing the necessary materials.

RESULT

Need assessment was done by research team to identify community knowledge about malaria infection and their capacity in an effort to control malaria infection based on risk factors. Need assessment conducted by Rural Appraisal Participatory procedures involving village communities. Through these activities got result that most of the people do not understand the aspects of malaria causes, modes of transmission and means of prevention. There are temporary communities who argued that frequent fever caused by eating spoiled food or caused by fatigue.

Most people do not know that the mosquito is a malaria vector and avoid mosquito bites as well as managing the environment is necessary to prevent malaria infection. Health education on malaria prevention was given to raise people malaria infection awareness and knowledge. Transect a procedure which was carried out jointly with the community leaders to identify mosquito breeding places and habits of people at risk of contracting malaria infection.

Participants were taught to practice simple socio dramas to be used as a method generates an awareness of the dangers and financial losses caused by malaria infection. It was very necessary because people were used to live with malaria diseases. Malaria sign and symptoms are no longer regarded as an infection tremendous problem. In order to enhance economic capability we trained them to make “jenang salak” since this local fruit is very abundant and available at the time of the harvest season.

Based on the results of need assessment, capacity building for community members and village officials was conducted to prepare them in facilitating the community to recognize problem, identify and delivering solution by utilizing local resources. Twenty persons involved in this activity. They were Health center personnel, village personnel, Women Welfare Organization, prominence persons, and health Cadres.

Trained people from local place built a group containing village member to discus about malaria in term of disease and factors related to its transmission. Commitment rose to be applied in each village. Community member stated the schedule of activities whilst health and county personnel play their role as support team, distributing the certain material for instance bed net, *kepala timah* fish to be cultivated in ponds as mosquito larva predator, poster and serial sermon materials (*khobah jumat)* containing malaria awareness made by spiritual leader which was involved in community participation activity. One hundred thirty two books were distributed to mosque to be used by spiritual leader.

We trained people to use bed net to protect people from mosquito bite. A small number of bed nets were provided as a stimulant. Local people decided by themselves who would get this bed net stimulant. Community member who got the stimulant will collect some amount of money every week to buy another bed net for other community member, by then all of community members would have their own bed net.

Another activity was environment awareness. In Sigeblog village, there were many ponds surrounding family houses. Health education brought a new knowledge of community member to prevent this pond to be mosquito breeding place. They were committed to clean regularly and cultivate “ikan kepala timah” as mosquito larva predator. So far 128 ponds were cleaned and poured with fish.

In economic generating activity, we facilitated community members to produce “jenang salak” with local fruit, *salak* as main material. A simple finance management was introduce to be practiced by family members. Four groups of family member participated and run the family scale economic generating a ctivity.

DISCUSSION

Community empowerment refers to the serial processes to enable people to control over their lives. In this case, empowerment has a meaning as deliberate activity, planned to increase people awareness and capacity to recognize problem and find solution for their better lives. Empowered people and community will voluntarily engage to a certain activity as result of their own dynamic process. This method allowing local people becomes a subject instead to be an object of activity and take offer role to lead of activities. Outside people such as researchers may take role as facilitating of the process (Baum, 2008).

Community empowerment therefore is more than the involvement, participation or engagement the community. It implies the social change and shows that community is the owner of activities. External agent just catalyze, facilitating community to acquaintance their own power and resources. Participatory approach would let community members shift role from just being object of activity to be the subject of activity (Baum, 2008). Participatory approaches in communication will encourage discussion and increased knowledge and awareness of problems (Katz, 1997). The

critical thinking enables communities to understand the interplay of forces operating on their lives, and helps them take their own action.

This study showed that once we handed authority to community, they will show their power to manage action. It recognizes that if some people are going to be empowered, then others will be sharing their existing power and giving some of it up. The ownership of power is the central concept in community empowerment. Empowered community would recognize their own capability to generate and sustaining problem solving activities (Labonte, 2008; Green, 2001).

Community empowerment necessarily addresses the social, cultural, political and economic determinants that underpin health (Baum, 2008), and seeks to build partnerships with other sectors in finding solutions. In our study, we facilitated by utilizing existing social dynamics in the community. Invited people to come and discuss in the way they used to do. Respect with local wisdom and understand their way of life. We also facilitated people to generate family scale economic generating. These effort demonstrated success in getting community participation to start their activity to solve health problems. Communication plays a vital role in ensuring community empowerment.

CONCLUSION

After facilitating this activity we learned that community members have their own capacity to solve problem. Once we let them to participate, they will use the local wisdom to view problem and find solution using local resources. Community participation approach proved superior to sustain malaria prevention activity.

Integrated approach by involving others sectors, health, village government, religious leader, and community members brought better result.

REFERENCES

Baum. F, 2008, The New Public Health, third edition, Oxford University Press.

Glanz, K, Rimer, BK, Lewis FM, 2002, Health Behavior and Health Education. Theory, Research and Practice. San Fransisco: Wiley and Sons

Green. L.W, 2001, Health Promotion in Practice, Mayfield Press.

Katz. J, Peberdy. A, 1997, Promoting Health, knowledge and practice, Open University Press.

Labonte. R, 2008, Health Promotion in Action, from global to local, Palgrave.

Suhartono et al,2001, Malria risk factors study in Central Java Province, ICDC Project.

THE EFFECTIVENESSOF CITRONELLA (*Cymbopogonnardus*L.) MICROCAPSULES AS LARVICIDES AGAINST *Aedesaegypti* LARVAE

MutiaraWidawati, M. Umar Riandi
Unit of Health Research and Development on Vector-Borned disease control
Ciamis- Pangandaran Indonesia
Corespondence: mutiara_w61@yahoo.com

ABSTRACT

Background: Vector control of dengue fever can be accomplished by reducing the potential for mosquito breeding places of *Aedes aegypti*. One of efforts to reduce breeding places is using a natural larvicide made from essential oils of citronella (*Cymbopogon nardus* L.). The content of Citronella leaf extract can act as a stomach poison and respiratory toxins that can lead to death of larvae. This study aimed to find the effectiveness of modified larvicides of Citronella scented essential oils in microcapsules form against third instar larvae of *Aedes aegypti*.
Method: This was an experimental using a completely randomized design with 6 treatment groups. Each group contained 25 third instar larvae of *Aedes aegypti* and the procedure were repeated 4 times. Concentrations of essential oil in the microcapsules are 10, 50, 100, 200, 400 and 500 ppm respectively, with Temephos 1 % as a positive control. The study was conducted in Health Research Laboratory of LokaLitbang P2B2 Ciamis, Pangandaran 2013. Data was analyzed using Kruskall Wallis test and Mann Whitney post hoc test to determine differences between concentrations.
Results: Concentrations ranging from 10 to 500 ppm, at 350 ppm, the larvae were dead by 99 % after contact for 24 hours. Based on the Mann - Whitney test, the effectiveness of Citronella scented microcapsules do not have significant differences with Temephos. Based on data durability, 350 ppm microcapsules were active and still have value above 90 % after 20 days.
Conclusion: Based on these results, it was concluded that the microcapsules scented Citronella were potential to be larvicides of *Aedes aegypti* larvae.
Keywords: microcapsules, essential oil, Citronella, *Cymbopogon nardus* L., biolarvicide.

BACKGROUND

Dengue is a disease which is transmitted by the *Aedesaegypti* mosquito vector [1]. These vectors breed in places where there iswater [2]. In order to reduce the impact of stagnant water as breeding places of *Ae.aegypti*, an insecticide in the form of larvicides is needed [3]. In the environment, the toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides [4], and for more detailed studies of naturally-occurring insecticides [5].Although synthetic pesticides have an effect on the target [6], but the treatment is conducted continuously and it may lead to a variety of threats both resistance [7] and collecting non-biodegradable toxins in our ecosystem [8].

Plants are the most suitable alternative form to be used as a natural insecticide [9]. Plants have a wide range of active compounds as a vector control [8]. The potential of volatile oils extracted from turmeric, citronella grass and hairy basil as topical repellents against both day- and night-biting mosquitoes has been demonstrated and recommended as a variant of the material for bio insecticide, especially for mosquitoes [10]. The chemical composition of citronella oil was studied and it was found that the crude essential oil consists of active ingredients that markedly suppressed the growth of several species of Aspergillus, Penicillium and Eurotium. The most active compounds among the 16 volatiles examined in Citronella oil, consisting of 6 major constituents of the essential oil and 10 other related monoterpenes, were citronellal and linalool[11].

Some essential oil and its components have a repellent and larvicidal properties [12]. The compounds in the essential oils like: camphor, cineole, methyl eugenol, limonene, myrcene and thymol have nature resist insects, and also can stop the activities of larvae [13].Consoli et al. have investigated the effects of monoterpenoid essential oil for a few cockroaches and house flies [14] and linalool were identified as inhibitors of acetylcholinesterase [15].

Citronella essential oils are highly volatile [16], especially in tropical regions such as Indonesia [17]. We need a formulation that can control the volatile essential oil so that it can be released slowly at a constant speed.Controlled delivery may be defined as a technique or method in which active chemicals are made available to a specified target at a rate and duration designed to accomplish an intended effect [18].

Controlled release by polymeric adduct formation is one of the best methods so far commercialized. Here, monomer or prepolymer or oligomers are used for adduct formation with active agent by cross linking reactions. Selection of cross linker, time of cross linking and temperature of cross linking reactions are very much important for controlling the rate of release of the active agent [19].

Sodium alginates (Na-Alg) is a material that wellknown for its advantage in forming soft matrices with CaCl2so that able to absorb sensitive material and having controlled release characteristic when cross-linked with glutaraldehide (GA), so we did the study of encapsulation with sodium alginate and glutaraldehide in order to create a larvicide that have long term durability and efficient The main purpose of this study is to test the effectivity of Citronellas essential oil in microcapsule form as a larvicidal against *Aedesaegypti* in laboratory study.

METHOD

This study is an experimental design research and using a completely randomized design with 6 treatment groups. Each group containing 25 third instar larvae of *Aedes aegypti* and the procedure were repeated for 4 times. Concentrations of essential oil in the microcapsules were 10 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm and 500 ppm with Temephos 1 % as a positive control. Furthermore, the data were analyzed by using the Kruskall Wallis test and Mann Whitney post hoc test to determine differences between concentrations.

Micro-encapsulated Citronellas oil: Sodium alginate beads containing Citronellas oil were prepared by mixing Citronellas oil in a sodium alginate solution followed by dropwise addition of this solution into methanol containing glutaraldehyde (1%) and HCl (1% of 1 N) solution under constant stirring [20]. The beads were separated from

methanol at different time intervals, washed with water and dried.UV-visible spectrophotometric methods were used to study the encapsulation efficiencies and controlled release behavior of encapsulated Citronellas oil.

RESULT

Mean diameters, encapsulation efficiency, oil content, and oil load of piper Citronellas oil encapsulated are shown in Table 1.

Table 1. Mean diameters, span size distribution, rate oil/polymer, and encapsulation efficiency (EE) of microcapsules formulation

Size	Encapsulation efficiency	oil content	oil load
0,48 mm	88%	63%	73%

The particles presented mean diameters of 0.48mm. Unlike the other microcapsule, this capsule is rather large in size, presumably because we use the pump in the making of those capsules. The capsules showed no oils phase separation during 30 days of study, on the other hand, the dilutions of Citronellas oils using a surfactant solution of 0.1 % tween showed the presence of a thin layer of oil on the surface of the solution after 2 days of preparation, which is indicating phase separation, meanwhile the micro capsules can be dispersed easily in water without any additives. Mortality Data of larvae from the experiment can be seen in Table 2

Table 2. Average percentage of Aedes aegypti larvae mortality from various concentration in 4320 minutes (72 hours)

Concentration	Larvae mortality (%) by minutes										
(ppm)	5	10	20	40	60	120	240	480	1440	2880	4320
10	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	1	1	1	1	1	13	60
100	0	1	1	1	1	5	19	29	34	56	66
200	0	1	1	1	1	7	20	31	50	87	91
400	0	1	1	1	7	43	53	59	99	99	99
500	0	1	1	1	7	43	53	60	100	100	100
control +	0	0	0	3	8	94	98	100	100	100	100

Data were analyzed to determine differences in treatment at each concentration. Normality test results showed that larval mortality data is not normal, so it cannot be analyzed further by using one way ANOVA. Hence, the data were analyzed by the Kruskal-Wallis test, based on the test results obtained $p < 0.05$ thus concluded that there are significant differences in mortality between the 2 concentrations of larvae. After that, the post hoc Mann Whitney test were done to determine the concentration of the most significant and can cause larval mortality ($p < 0.05$), the results of the Mann-Whitney test data can be seen in Table 3.

Table 3. The comparative statistic test between groups
(Post-hoc Mann-Whitney analysis)

Concentration	10	50	100	200	350	400	500
10	-						
50	0.014	-					
100	0.014	0.02	-				
200	0.014	0.02	0.02	-			
400	0.014	0.02	0.02	0.023	0.132	-	
500	0.014	0.02	0.02	0.023	0.132	0.023	-
Control+	0.009	0.014	0.014	0.047	0.132	0.132	0.132

(*) significant difference with alpha 5%

At 400 ppm, the larvae were dead by 99 % after contact for 24 hours. Based on the Mann - Whitney test, the effectiveness of Citronella scented microcapsules do not have significant differences with Temephos. In order to determine the LC50,probit analysis were used. Based on Table 3 LC50 values obtained from minute to minute late start decreasing.It can be seen that increasing the concentration, the higher the percent mortality of larvae.

Table 4. LC50 of Aedes aegypti larvae in various observation time

No.	Time (minute)	LC50
1	5	-
2	10	-
3	20	-
4	40	-
5	60	1874,325
6	120	412,226
7	240	217,025
8	480	195,837
9	1440	175,6
10	2880	175,6
11	4320	175,6

DISCUSSION

According to WHO [21], the concentration of larvicides considered effective if the test can cause larval mortality between 10-95 % which will be used to search for lethal concentration values.According to the Mann-Whitney test , the test between the positive control with concentrations of 350 ppm produced p values >0.05 which is statistically stated that the positive control and the treatment did not have significant differences.The durability of Citronella oil and microcapsules is shown in

Figure *1*.

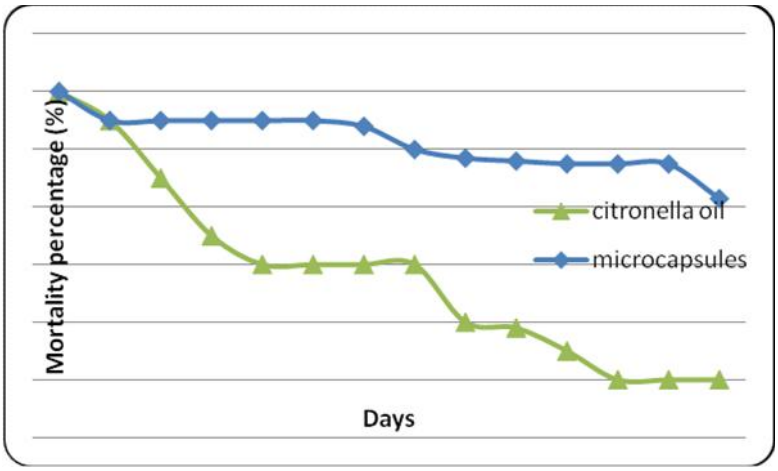

Figure 1 Durability of Citronella oil and its microcapsules

The residual larvicidal activity of solutions containing 350 ppm microcapsules was remained above 90% for 12 days. For the oil, a rapid reduction is seen at the second and the 16th day and keep declining until 0% in 24th day, meanwhile microcapsules is also declining but until the 24th day, the larval mortality remain to be above 70%.

The residual activity time of microcapsule was greater than pure oil and this property is proved to be superior in microcapsules. In solution that contained oil solution at concentrations 350 ppm, the formation of a thin layer of oil was showed on the surface of the solution containing the larvae that observed during the study. This is caused by the physical instability of the solution, and that may have facilitated oilconsumption by larvae during respiratory process. The solution surface is a factor that can affect not only larval developmentbut also other aquatic organism respiratory systems [22]. This problem may bother the environmental balance.The results of this study are consistent with research conducted by Phasomkusolsil [23], which states that citronella essential oil can kill mosquitoes within 24 hours .

CONCLUSION

Based on these results, it was concluded that the microcapsules with 350 ppm concentration of scented Citronella were potential to be larvicides of *Aedes aegypti* larvae. The microcapsules meet the requirement of good larvicides based on WHO which stated that good larvicides have its LC with 90% mortality.

REFERENCES

1. Tennyson S, Samraj DA, Jeyasundar D, Chalieu K, College MC, Nadu T. Larvicidal Efficacy of Plant Oils Against the Dengue Vector Aedes aegypti (L .) (Diptera : Culicidae). *Middle-East J. Sci. Res.* 2013;13(1):64–68.
2. Vadeyar LK, Hooli AA, Kallapur SNHS V. Bioefficacy of Vinca rosea leaf powder on Aedes aegypti L . *J. Biopestic.* 2010;3(1 Special Issue):189–191.
3. Lam SK. Strategies for Dengue Control in Malaysia. *Trop. Med.* 1994;35(4):303–307.

4. Macedo M, Consoli R, Grandi T, et al. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against Aedes fluviatilis (Diptera: Culicidae). *Mem Inst Oswaldo Cruz*. 1997;92(2):565–70.
5. Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan a. Ovicidal and repellent activities of botanical extracts against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.* 2011;1(1):43–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3609154&tool=pmcentrez&rendertype=abstract. Accessed November 12, 2013.
6. Walton WE, Eldridge BF. Pest Notes. 2009;(October):1–5.
7. Harris AF, Rajatileka S, Ranson H. Pyrethroid resistance in Aedes aegypti from Grand Cayman. *Am. J. Trop. Med. Hyg.* 2010;83(2):277–84. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2911171&tool=pmcentrez&rendertype=abstract. Accessed November 6, 2013.
8. Kihampa C, Joseph CC, Nkunya MHH, et al. Larvicidal and IGR activity of extract of Tanzanian plants against malaria vector mosquitoes. *J. Vector Borne Dis.* 2009;46(2):145–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19502695.
9. Ruikar AD, Pawar P V, Sen A, Phalgune UD, Puranik VG, Deshpande NR. Larvicidal potential of Mimusops elengi against Aedes aegypti (L) and Culex quinquefasciatus (Say). *J. Vector Borne Dis.* 2012;49(2):111–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22898484.
10. Tawatsin A, Asavadachanukorn P, Thavara U, et al. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against Aedes aegypti (Diptera: Culicidae). *Southeast Asian J. Trop. Med. Public Health*. 2006;37(5):915–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17333734.
11. Nakahara K, Alzoreky N, Yoshihashi T, Al E. Chemical composition and antifungal activity of essential oil from Cymbopogon nardus (citronella grass). *JARQ*. 2003;37(4):249–252.
12. Krishnappa K, Elumalai K, Dhanasekaran S, Gokulakrishnan J. Larvicidal and repellent properties of Adansonia digitata against medically important human malarial vector mosquito Anopheles stephensi (Diptera: Culicidae). *J. Vector Borne Dis.* 2012;49(2):86–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22898480.
13. Wahyuni D. Larvicidal Activity of Essential Oils of Piper betle from the Indonesian Plants against Aedes Aegypti L. *J. Appl. Environ. Biol. Sci.* 2012;2(6):249–254.
14. Consoli R, Mendes N, JPP SB de S, Lamounier M. Influência de diversos derivados de vegetais na sobrevida das larvas Aedes fluviatilis (Lutz) (Diptera: Culicidae) em laboratório. *Mem Inst Oswaldo Cruz*. 1988;83:87–93.
15. Ryan M, Byrne O. Plant insect coevolution and inhibition of acetylcholinesterase. *J. Chem. Ecol*. 1988;14(8):65–75.
16. Rani N, Wany A, Vidyarthi AS, Pandey DM. Study of Citronella leaf based herbal mosquito repellents using natural binders. *Curr. Res. Microbiol. Biotechnol.* 2013;1(3):98–103.
17. Daouk RK, Dagher SM, Sattout EJ. Antifungal activity of the essential oil of Origanum syriacum L. *J. Food Prot.* 1995;58(10):1147–1149.

18. A F. *Controlled Release Technology-Methods, Theory and Applications*. Vol.1 ed. (Boca Raton, ed.). Florida: CRC Press Inc.; 1980:2.
19. Devi N, Maji TK. Neem Seed Oil : Encapsulation and Controlled Release - Search for a Greener Alternative for Pest Control. In: Stoytcheva MD, ed. *Pesticides in the modern world-Pesticides Use and Management*. India: InTech; 2011:191–232. Available at: http://www.intechopen.com/books/pesticides-in-the-modern-world-pesticides-use-and-management/neem- seed-oil-encapsulation-and-controlled-release-search-for-a-greener-alternative-for-pest-control InTech.
20. Kulkarnia A, Soppimatha K, Aminabhavia T, Daveb A, Mehtab M. Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application. *J. Control Release*. 2000;63(1-2):97–105.
21. WHO. *Guidelines for laboratory and field testing of mosquito larvicides*.; 2005:WHO/CDS/WHOPES/GCDPP/2005.13.
22. Kanis LA, Prophiro JS, Vieira EDS, et al. Larvicidal activity of Copaifera sp. (Leguminosae) oleoresin microcapsules against Aedes aegypti (Diptera: Culicidae) larvae. *Parasitol. Res.* 2012;110(3):1173–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21850452. Accessed November 12, 2013.
23. Phasomkusolsil S, Soonwera M. Potential Larvicidal and Pupacidal Activities of Herbal Essential Oils Against Culex quinquefasciatus Say and Anopheles minimus (Theobald). *Southeast Asian J Trop Med Public Heal.* 2010;41(6).

VECTORIAL CAPACITY, ENTOMOLOGICAL INOCULATION RATE, AND STABILITY INDEX OF *Anopheles sundaicus* (L.) IN SUKARESIK VILLAGE PANGANDARAN WEST JAVA

Pandji Wibawa Dhewantara, Heni Prasetyowati, Dewi Nur Hodijah, Asep Jajang Kusnandar
Lokalitbang Pengendalian Penyakit Bersumber Binatang Ciamis
Correspondence: p.dhewantara@gmail.com

ABSTRACT

Backgrounds: Malaria remains as potentially re-emergent disease in Sukaresik village, Pangandaran, West Java. Information of vectorial capacity, entomological inoculation rate (EIR), and stability index of well-known malaria vector in this area, *Anopheles sundaicus*, was limited and rarely published. An advanced analysis of spot-survey result in 2011 was carried out to determine those entomological-malaria related indices.
Method: ELISA test was assigned to 99 adult mosquitoes *An. sundaicus*which collected from 10x12-h human landing collection in order to identify blood-meal type and detect *Plasmodium*circum-sporozoit.
Results: Based on ELISA results, the anthrophofilic proportion was 56.10 % and positively contained *P.vivax,* with sporozoit index was 15.15 %. From ten times adult collection, density of *An. sundaicus*was 1.98 bites per night; daily survival rate was 0.871; and expectation of infective life up to 7.246 days.
Conclusion: Vectorial capacity of *An. sundaicus* was 2.736; EIR up to 29.08 %; with vector stability index 1.425.
Keywords: vectorial capacity, EIR, stability index, *Anopheles sundaicus*, Pangandaran

BACKGROUND

Malaria, which caused by *Plasmodium* and transmitted by Genus *Anopheles* mosquito, is one of the infectious diseases that still to be considered in Sukaresik village, Pangandaran, south coast of West Java, Indonesia. From ecological perspective, previous study indicated that environmental characteristics of Sukaresik village were suitable for malaria vector development, such as *Anopheles sundaicus*. In 2008, 6 malaria cases were found (API 4.56 per mil) and 3 indigenous cases in 2011 was reported [1]. Malaria infection emerged as a result of contact between blood-feeding infective *Anopheles* mosquitoes and human. Sporozoite-stage parasites inoculated by even a single infectious mosquito could infect human and would be life-threatening [2].

In order to determine degree of malaria transmission, alternative approaches based on vectorial capacity (C) and entomologic inoculation rates (EIRs) index measurement could be undertook. Vectorial capacity and EIRs are a quantitative entomological indicator that useful to describe malaria epidemiology status in area [3]. The vectorial capacity is a measure efficiency of pathogen transmission, while EIRs is a key factor when establishing the direct estimation the transmission risk. A vectorial capacity value of 0.01 reflects the vector capability to maintain malaria transmission in

ecosystem [4]. While, an area with EIRs lower than 1 is considered as a hypoendemic zone, while EIRs between 100 to 1,000 characterized a holoendemic zone [5]. The EIRs indicates number of infective bites that an individual receives during a determined period of time. It could be measured by adult Anophelines collection when they land on man who acts as bait [6].

The rate at which malaria is transmitted from infected people through the mosquito population to infect others is determined by the proportion of persons infectious to mosquito at any one time, the numbers and survival rate of the mosquitoes, their tendency to bite humans, the duration of each feeding cycle, and the length of time taken for the parasite to develop in the mosquito (the extrinsic incubation period) [7]. Even *An. sundaicus* have been confirmed as important vector of malaria in Indonesia since 1950's, particularly on coastal ecosystem along Java Island [8.9], data on malaria epidemiological indicators of this species, were very limited. Few studies about *An. sundaicus* vector capacity and EIRs also had been reported from particular area in Indonesia and South East Asian countries [10,11].

Aim of this study was to provide detail of entomological indicators in relation with current malaria transmission in Sukaresik village. An advance study to measure vectorial capacity, entomological innoculation rate, and vector stability index of *An. sundaicus* was assigned based on its density and longevity data from another part of research in 2011.

METHOD

A spot-survey was conducted in Sukaresik village, sub-district of Sidamulih, Pangandaran, West Java (07°40'47.63"S; 108°35'16.37"E) from September-November 2011. Mosquito collection was conducted during 10x12-hours by six collectors in three houses, both indoor-outdoor human landing and resting method capture [6]. For this research, ethical clearance was obtained from Ministry of Health of Indonesia. In another part of this study, up to 1,430 individuals of *An. sundaicus* was captured through human landing collection; each of 418 individuals (indoor) and 1,012 individuals (outdoor). While, 872 individuals captured from resting collection, respectively. Density and longevity measurement of *An. sundaicus* in the same place has been reported [1]. Field specimens of *An. sundaicus* were individually places in tubes containing silica gel dessicant and cotton immediately after collection and stored at refrigerator until analyzing.

Polymerase chain reaction assay was assigned to identify bloodmeal type and detect *Plasmodium* sporozoite on 99 individuals of *An. sundaicus.* For all mosquitoes, heads/thoraces were separated from abdomens and only the DNA isolated from engorged abdomens were used in the blood meal diagnostic analysis. Abdomens of the blood-fed females were ground and analyzed for blood meal sources against human, dog, and cow primers. Bloodmeal analysis using cytochrome b-based multiplex PCR following Kent'smethod [12]. Presence of *P. vivax* and *P. falciparum* in *Anopheles* specimens was also determined by PCR according to Singh *et.al* procedures [13]. Polymerase chain reaction tests were conducted at Eijkman Institute for Molecular Biology, Jakarta.

Human blood index (HBI) was calculated by dividing number of *An. sundaicus* with human blood with total number of *An. sundaicus* with blood. While sporozoite rate

was calculated by dividing number of *An. sundaicus* contains *Plasmodium* with total number of *An. sundaicus* tested. Then, vectorial capacity (C) index expressed by mathematical formula as follow:

$$C = \frac{ma^2p^n}{-\ln p}$$

(m = density of vector in relation to man (bites/person/night); a = number of blood meals taken on man per vector per day (= human blood index multiplied by 0.5, if a gonotrophic cycle of two days is assumed); p = daily survival rate; n = incubation period [6,14]).

Entomological inoculation rates (EIRs) was determined to measure intensity of malaria transmission. EIRs was calculated by multiplying man-biting rate (ma) and proportion of sporozoite rate (s) [2,6]. The stability index (SI) expressed by formula, SI $=\frac{a}{-\ln p}$, which represents the average of bites taken on man by one mosquito in its whole lifetime and it would indicated vector stability on malaria transmission in particular area [15].

RESULT

Ninety nine individuals were tested through polymerase chain reaction, both to identify bloodmeal type and parasite. Number of 35 bloodfed *An. sundaicus* specimens were diagnosed for bloodmeal analysis. Results showed that 21 individuals contained human blood; and cow, goat, other mammals. The proportion of human-blood was 60%. Furthermore, 12 from 99 individuals were identified positively containing *P. vivax* (Pv210). None of *P. falciparum* was detected from specimens. Each specimen was captured from indoor collection (4 individuals), outdoor (4 individuals), wall resting (3 individuals); and unidentified collection (1 individual). The sporozoite rate was 12.12 %.

In addition to calculate vectorial capacity, entomological inoculation rate, and stability index of *An. sundaicus*, data (ie. man-biting rate, days of infective life) from another part of this study was referred. The man-biting rate and infective life expectancy of *An. sundaicus* was 1.98 bites/person/night and 7.246 days, respectively.[1] As the result, the vectorial capacity of *An. sundaicus* was 2.869; entomological inoculation rate was 0.291 infective bite per person per night; and stability index of *An. sundaicus* was 1.45. The calculation of these three indicators summarized in Table 1.

The period of sporogony (n) differs according to the species of *Plasmodium* and varies with the mean temperature of the environment. Sporogonic periods of 8-9 days in *P. vivax* and 11-12 days in *P. falciparum* are normal at mean temperatures of 26°C-27.5°C. It is also can be calculated by 111/T-16 for *P. falciparum* and 105/T-14.5 for *P. vivax* (where T = mean temperature) [14,15].

Table 1. Calculation of Vectorial Capacity, Entomological Inoculation Rate, and Stability Index of *An. sundaicus* in Sukaresik, Pangandaran, West Java

Index	Value
Mean man-biting rate (m)[a]	1.98
Man-biting habit (a)[b]	0.2
Infective life (days)[c]	7.246
Sporozoite rate (s)	0.1212
Vectorial Capacity $\left(\frac{ma^2 p^n}{-\ln p}\right)$	2.869
Entomological Inoculation Rate (*ma.s*)	0.291
Stability Index $\left(\frac{a}{-\ln p}\right)$	1.449

[a,c]based on Dhewantara et al. [1]

[b]obtained from human blood index (HBI) = 0.60 and 3-days gonotropic cycle.

DISCUSSION

Considering malaria as infectious and life-threatening diseases, so far, an effective integrated vector control is a crucial need. Entomological information is one of the important supporting data that could be utilized on implementing vector control. Some of entomological indicators are relevant to assess epidemiological status of malaria, including vectorial capacity (C), entomological inoculation rates (EIRs), and vector stability index.

Coastal ecosystem is where *Anopheles sundaicus* suitable to breed and develop. In Indonesia, it is widely distributed from Sumatra through Java to Bali [1,9]. Sukaresik village is geographically located in south coast West Java and environmentally-suited, beneficially to *An. sundaicus* development. Information on its vectorial capacity (C), entomological inoculation rate (EIRs), and stability index are strongly needed in order to determine status of malaria transmission, especially in Sukaresik village, Pangandaran.

Determining host preferences of mosquitoes is necessary to understanding their probability as vectors of diseases. This research showed that *An. sundaicus* in Sukaresik village has preferences on human (antropophily). It showed by relatively high proportion of human-blood index (HBI = 60%). This behavior is potential in transmitting parasite *Plasmodium* to human. However, there were still many factors that determined malaria transmission, i.e.vector density, longevity, and some environmental properties. Sporozoite detection assay showed that *An. sundaicus* have ability to be a host of *Plasmodium vivax.* This receptivity might be influenced by their genetic. Mosquito's receptivity to parasite infection indicates physiological suitability between mosquitoes and host [4,16].

In our research, vector capacity of *An. sundaicus* in Sukaresik village was higher than 0.01. It could be indicated that this species represents capability on maintaining endemicity of malaria in ecosystem. Value of vector capacity (0.01) is minimum requirements to vector so that it could be maintained malaria transmission in area [4].

Based on its formula, vector capacity strongly depends on density, biting frequency, longevity, and also susceptibility to *Plasmodium* infection. On previous study, some of these factors have addressed.

To determine effectiveness of malaria transmission to human, EIRs could be represented. Our research showed EIRs of *An. sundaicus* in Sukaresik was up to 0.291 infective bites per person per night. It was equals to 106.2 infective bites per person per year. The EIRs was higher than EIRs reported in coastal area in Rayong Province, Thailand (76.6 positive bites/person/year) and Nongsa Pantai Village, Batam City, Riau [10,11]. An earlier study was reported that substantial reductions in malaria prevalence are only likely to be achieved when EIRs are reduced to levels less than 1 infective bite per person per year. In endemic areas where environmentally suitable for malaria transmission and EIRs were relatively high, a new tools for malaria transmission control could be used in conjunction with already proven measures, i.e insecticide-treated bed nets [2]. EIRs between 100 to 1,000 characterized a holoendemic zone [5].

Stability index is another entomological indicator to describe species stability who can act as a vector. Our study represented stability of *An. sundaicus* as malaria vector in Sukaresik village (SI > 0.5) [15]. The stability index of *An. sundaicus* was 1.45, indicated that this species still has enough stability as malaria vector in this village. Therefore, this condition directly suggested that vector control programs must be evaluated and revitalized to modify stability index and reduce malaria transmission.
Our research limitations are also should be considered. Value of these three indicators might be over-estimated, because of limited *An. sundaicus* specimens tested and short period of mosquito survey (3 months and 10 times). A longitudinal survey throughout the year is recommended to obtain better values and conclusions. However, this result might be as basic and useful information for early warning detection of malaria in Sukaresik village and encourage integrated vector control management implementation.

CONCLUSIONS

Three entomological indicators of *Anopheles sundaicus*, i.e vectorial capacity, entomological inoculation rate, and stability index concluded that this Anopheline species still represented great potential on transmitting malariain Sukaresik village, Pangandaran, West Java. Evaluation and revitalizing integrated vector control management implementation were strongly encouraged to reduce transmission risk of malaria.

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REFERENCES

1. Dhewantara PW, Astuti EP, Pradani FY. Studi Bioekologi Nyamuk Anopheles sundaicus di Desa Sukaresik Kecamatan Sidamulih Kabupaten Ciamis. *Bul. Penelit. Kesehat.* 2013;41(1):26–36.
2. Beier JC, Killeen GF, Githure JI. Short report: entomologic inoculation rates and Plasmodium falciparum malaria prevalence in Africa. *Am. J. Trop. Med. Hyg.* 1999;61(1):109–13.
3. Garrett-Jones C. the Human Blood Index of Malaria Vectors in Relation To Epidemiological Assessment. *Bull. World Health Organ.* 1964;30:241–61..
4. Gilles HM W DA. Epidemiology of malaria. In: Arnold E, ed. *Bruce-Chwatt's essential malariology 3rd edition*. London Boston Melbourne Auckland; 1993:131–139.
5. Cano J, Descalzo MA, Moreno M, et al. Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). *Malar. J.* 2006;5:21.
6. WHO. *Malaria entomology and vector control, Learner's Guide*. Geneve: World Health Organization; 2003.
7. P. M. Graves, T. R. Burkot, A. J. Saul RJH and RC. Estimation of Anopheline Survival Rate, Vectorial Capacity and Mosquito Infection Probability from Malaria Vector Infection Rates in Villages Near Madang, Papua New Guinea. *J. Appl. Ecol.* 1990;27(1):134–147.
8. Kirnowardoyo S. Status of Anopheles malaria vectors in Indonesia. Southeast Asian J Trop Med Public Health 1985, 16(1):129-32. *Southeast Asian J Trop Med Public Heal.* 1985;16(1):129–132.
9. Ndoen E, Wild C, Dale P, Sipe N, Dale M. Relationships between anopheline mosquitoes and topography in West Timor and Java, Indonesia. *Malar. J.* 2010;9:242. Available at:
10. Susanna D. Entomological Inoculation Rate (EIR) of Malaria Transmission in Coastal Ecosystem in Nongsa Pantai Village, Batam City, Indonesia. Available at: www.ifeh2012.org/get.php?f.309. Accessed October 22, 2013.
11. Sumruayphol S. *Anopheles sundaicus s.l. population dynamics, species complex and insecticide susceptibility in a coastal area of Rayong Province. [Thesis]. Thailand*.; 2009.
12. Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am. J. Trop. Med. Hyg.* 2005;73(2):336–42.
13. Singh B, Bobogare a, Cox-Singh J, Snounou G, Abdullah MS, Rahman H a. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *Am. J. Trop. Med. Hyg.* 1999;60(4):687–92.
14. Garrett-Jones C, Shidrawi GR. Malaria vectorial capacity of a population of Anopheles gambiae: an exercise in epidemiological entomology. *Bull. World Health Organ.* 1969;40(4):531–45.
15. Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. A global index representing the stability of malaria transmission. *Am. J. Trop. Med. Hyg.* 2004;70(5):486–98.
16. N. Becker et al. *Mosquitoes and Their Control*. 2nd Ed. London, UK: Springer-Verlag Berlin Heidelberg; 2010.

ACTIVITY OF MODIFIED CITRONELLA REPELLENT GEL AGAINST *Aedes aegypti*

Muhammad Umar Riandi, Mutiara Widawati
Research and Development Unit of Animal-Borne Diseases, Ciamis
Email: mutiara_w61@yahoo.com

ABSTRACT

Background: *Aedes aegypti* is a dengue fever vector that has a fairly high incidence in Indonesia. Repellent are widely use to prevent *Aedes aegypti* bite personally. Repellent with active chemical constituent have negative consequences for human health and potentially make mosquito's resistance to the substance. As a solution to this problem, repellent that is made from natural ingredients are uses. The aims of this study were to determine the protective level of citronella (*Cymbopogon nardus*) gel with the addition of fixative substance patchouli (*Pogostemon cablin*) oil as repellent for *Aedes aegypti* mosquito.
Method: The research was conducted in the health research laboratory of Loka Litbang P2B2 Ciamis. This was an experimental study with a randomized block design. Test materials consisted of commercial DEET lotions, Citronella fixative gel and Citronella gel without fixative substance. Each test consists of 6 groups of observation time. Protection level was analyzed using ANOVA.
Results: The average repellencies of DEET, Citronella fixative gel, and Citronella gel without fixative substance are 91.02%, 88.72%, and 82.66% within 6 hour observation. This result did not show any significant differences.
Conclusion: Both formulations have the potential to be an effective repellent.
Keyword: repellent, *Cymbopogon nardus*, *Aedes aegypti*, *Pogostemon cablin*

BACKGROUND

Dengue hemorrhagic fever (DHF) has become a public health problem for more than 41 years in Indonesia. Since 1968 there has been an increase in the number of dengue endemic provinces and districts / cities, from 2 provinces and 2 cities, to 32 (97%) and 382 (77%) districts/cities in 2009. Maluku province, from 2002 until 2009 there were no dengue cases reported. In addition there is also an increase in the number of dengue cases, from 58 cases in 1968 to 158,912 cases in 2009 [1].

Dengue hemorrhagic fever (DHF) causes by a member of the genus flavivirus dengue virus which is transmitted by the mosquito *Aedes* vectors. As a vector for DHF in Indonesia, *Aedes aegypti* and *Aedes albopictus* is the main and secondary vector. The mosquito species have anthropophilic nature, preferring human blood than animal blood. *Aedes* mosquitoes are also multiple feeding, the need of blood were usually satisfied by several biting in one gonothropic cycle. The nature of dengue increased risk of transmission in more densely populated residential areas. One infective mosquito over a bites period of time will be able to transmit the virus to more than one person [2].

Several methods of vector control has been widely known and used by dengue control program are: 1. Environmental management, 2. Biological Control, 3. Chemical

control, 4. Community participation, 5. Individual protection and 6. Law [2] To protect individually from the risk of dengue transmission can be done by using repellent or wear clothing that reduces mosquito bites [2,3].

Repellent that are widely known contain DEET (N, N-diethyl-m-tolumide) 10-12.5%. DEET is a harmful toxic chemical. DEET is not soluble in water and stay in the skin for 8 hours. It systemically absorbed into the body through the skin blood circulation [4]. Only 10-15% of DEET can be wasted through the urine [5]. Government through *Komisi Pestisida* gives requirements which a repellent can be effective if it has protection power by 90% and can last for six hours [6].

Generally a natural repellent contain plants essential oils (volatile oil / essential oil), such as eucalyptus (*Malaleuca leucadendron* L), citronella (*Cymbopogon nardus* L), wintergreen (*Gaultheria procumbens* L), zodia (*Evodia suaveolens* Scheff), lavender (*Lavandula officinalis* Chaix), and others. Citronella oil has been widely used as repellent. Screened from 38 essential oils from plants against the mosquito *Aedes aegypti* under laboratory conditions using human subjects, the undiluted oils of *Cymbopogon nardus* (citronella), *Pogostemon cablin* (patchuli), *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* (Thai name: makaen) were the most effective and provided 2 hour of complete repellency [7].

Citronella essential oil gives a burning sensation when applied to the skin. Then gelling performed to this essential oil. Gel is a semi-solid system consisting of small inorganic particles suspense or large organic molecules penetrated by a fluid [8]. Gel form preparations is more widely used because of the cold feel on the skin, dries easily, form a continuous film that is easy to wash, and easy to use [9]. Gel preparation in this study, added with patchouli oil because it has a fixative effect and will make the scent last longer [10].

METHOD

The tools used in this study are: mosquitoes rearing equipment: scissors, plastic cups, trays, filter paper, pen, and a mosquito aspirator; gel making equipment: digital scales, watch glass, vaporizer bowls, measuring cups, water bath, glass beaker, magnetic stirrer, hot plate, pipette, mortar, erlenmeyer, stopwatch, stir bar, spatula, pH meter, Pycnometer, and refractometer.

Materials used in the study include insect repellent active ingredients, fixative materials, gel-forming material, and testing materials. Insect repellent active ingredients are essential oils wich is citronella oil and fixative materials used was patchouli oil, both obtained through distillation from Kebun Percobaan Manoko Lembang. Gelling material consist of carrageenan, distilled water, propilenglikol, and sodium benzoate. *Aedes aegypti* as test material obtained from Loka Litbang Pengendalian Penyakit Bersumber Binatang (P2B2) Ciamis.

Gelling formula based on research conducted by Anita Lukman [11] with modifications to the material for the addition of gel and addition of patchouli oil as much as much as 10% according to a study by G.C.Eka D and Hutagaol, L is effective as a fixative agen [12]. Formula can be seen in Table 1. Citronella oil mixed with patchouli oil and propylene glycol which has been weighed in erlenmeyer accordance to the formula, then stirred at 700 rpm for 10 minutes without heating (mixture 1). Carrageenan mixed separately with distilled water and stirred until become a

homogeneous mixture (mixture 2). Mixture 1 was added to the mixture 2 and then stirred quickly. Ethanol 96% was added gradually and then stirred until homogeneous.

Characterization of citronella gel

Organoleptic examination, including shape, odor, and color are conducted. Acidity examination (Martin et al. 1993) was performed with a pH meter. Instrument was calibrated with standard buffer solutions pH 4 and pH 7. Homogeneity examination was done by placing 1 g of the preparation on a piece of transparent glass, these stock must show a homogeneous structure and should not be seen any spots particles [13]. Gel physical stability examination conducted at room temperature. Tested gel is left for 2 months at room temperature. Weekly observation was done to determine whether there is a separation. Gel are not experiencing separation assessed as stable base [14]. Irritation test conducted with a patch test on human skin. Dosage of 1 g gel taken then smeared on the inner sleeve with 2 x 2 cm^2, covered with a bandage and adhesive tape were left for 24 hours. Observed symptoms such as redness and itching on the skin. Irritation test was conducted on 6 probandus [15].

Distribution test is determined by placing 0.5 g preparation carefully on graph paper that is coated on transparent plastic, left for 15 seconds and calculated the area given by the dosage then closed it again with plastic given a certain load 1 , 2 , and 5 g respectively, then left to stand for 60 seconds. The vast increase given by the dosage can be calculated [16]. Mosquito testing method performed based on Pesticide Commision [6]: 1 g repellent applied to the right hand skin and 1 g base gel applied to the left hand skin as control. Then put probandus both hands into the test cage that already containing 100 blood hungry female *Ae.aegypti* for 5 minutes, this hourly treatment was repeated 5 times. To determine effect of duration of the gel repellent properties, the test conducted until sixth hour (08:00 am to 02:00 pm). The number of landed mosquitoes is recorded.

The study was conducted at Loka Litbang P2B2 Ciamis laboratory. This study uses a randomized complete block design consisting of four group's comparison; 1 control treatment (base only smearing); 3 treatment trial: commercial repellent with 12.5% DEET (positive control), citronella gel, and citronella gel with fixative patchouli oil. Protection power is calculated by a formula [6]:

$$DP = \frac{K - R}{K} \times 100\%$$

DP: Protection Power
K: Amount of mosquito landed on control (gel base only)
R: Amount of mosquito landed on treatment

Reared 3-5 days old female *Aedes aegypti* on sugar diet are used for repellent protection power test. Results data obtained transformed to arcsin and analyzed with ANOVA test followed with Duncan test. Data processing was done by SPSS 17 software.

RESULT

Gel made form a thick white gel with strong odor. Acidity test showed ranged from 5.84 to 6.72. Based on homogeneity examination, after 2 months of storage at room temperature, gel still in homogeneous and stable form. Gel irritation test showed that citronella gel does not irritate probandus skin. Gel distribution test results showed that the addition of patchouli oil have no significant effect in increasing wider gel spread.

Table 1. Formulation of modiffied repellent

Material	Materialconcentration (%w/v)	
	Base	Formula
Citronellas oil	-	12
Pogostemons oil	-	1
Carageenan	5	5
Propilenglikol	15	15
Alcohol 96%	30	30
Aquadest	100	100

Distribution test of citronella gel with 1, 2, and 5 grams load showed dispersion results of 1.5453, 2.7261, and 4.8894 cm^2 respectively. The result is less compared to modified gel with fixative patchouli oil, 1.8756, 2.8894, and 4.9856 cm^2 respectively. But the dispersion difference between unmodified citronella gel and modified gel with patchouli oil fixation is not significant.

Protection power test result can be seen in Table 2. The results showed that both unmodified and modified citronella gel has a 100% protection at the first hour, but were reduced in the next hours. Different results were shown by DEET which began to decline in the fourth hour of repellent test.

Table 1 Protection Power within 6 hour against Ae. Aegypti

Treatment	Protection Power (%)						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
DEET	100.00	100.00	100.00	100.00	80.00	80.77	85.37
Citronella Gel	100.00	100.00	96.83	94.29	72.92	70.00	61.90
Modified Citronella Gel 1	100.00	100.00	98.67	96.15	76.32	80.00	80.60
Modified Citronella Gel 2	100.00	100.00	98.73	97.65	75.81	81.82	80.00
Modified Citronella Gel 3	100.00	100.00	98.65	97.50	75.44	80.36	81.67
Modified Citronella Gel 4	100.00	100.00	97.14	96.05	75.47	79.31	79.69
Modified Citronella Gel 5	100.00	100.00	100.00	97.10	77.27	79.25	81.03
Modified Citronella Gel Average	100.00	100.00	98.64	96.89	76.06	80.15	80.60

Table 3 show the average and standard deviation of protection in each group. Average DEET protection is 91.02%, the highest. While unmodified citronella gel is 82.66% and the average protection of citronella gel with patchouli oil fixation is 88.72%. ANOVA test concluded that there is no difference level of repellencies between three treatments at alpha 5%. Although there were no significant differences of the three experimental groups, but modified citronella gel with patchouli oil fixation shows 6.06% greater protection power than unmodified citronella gel at 82.66%.

Table 3. Protection from three groups of test after 6 hour

variable Protection (%)	Mean		
DEET	91.02	$\pm$	10.004
Citronella Gel	82.66	$\pm$	16.263
modified gel	88.72	$\pm$	10.882

DISCUSSION

Organoleptic examination of citronella gels including shape, color, and odor is done visually. According to Ketaren [17], essential oils have volatile properties even at room temperature. Although it does not decompose, essential oils will absorb oxygen from the air, resulting in changes in color, aroma, and viscosity that may cause changes in the chemical properties of the essential oils. Acidity of the gel made slightly acidic, but still within the range that can be tolerated by the skin, which is 4.5 to 7 [18]. In order to form a homogeneous gel, the concentration of ethanol used as a surfactant should range between 14-70% [19]. This study used 30% ethanol concentration accordance to formula based on research conducted by Anita Lukman [11]. Ethanol is a surfactant that helps Carageenan bond oil and water phase. Overall, modified gel can be used as a losion.

Research on activity of 38 plant essential oils conducted by Trongkit, Y *et.al*, found that nothing can prevent mosquito bites more than 2 hours [7]. This research data showed that both of unmodiffied and modiffied repellent are potential to be repellent, however, the effectivity of modiffied repellent is better when it was compared with unmodiffied repellent.

CONCLUSION

Based on the result, concluded that both citronella gel formulation have the potential to be mosquito repellent, however modiffied repellent turned out to be the only one that fulfilled the requirement to be an effective repellent.

REFERENCE

1. Buletin Jendela Epidemiologi. Pusat Data dan Surveilans Epidemiologi Kementerian Kesehatan R. *Vol. 2*. 2010;volume 2:43. Available at: http://www.depkes.go.id/downloads/publikasi/buletin/BULETIN DBD.pdf.
2. Sukowati S. Masalah Vektor Demam Berdarah Dengue (DBD) dan Pengandaliannya di Indonesia. *Bul. Jendela Epidemiol. Demam Berdarah Dengue*. 2010:26–30.
3. Sari W, Agustina E. UJI BIOEFIKASI MINYAK SERAI DAN PALA PRODUK KHAS ACEH SEBAGAI ANTI NYAMUK VEKTOR DEMAM BERDARAH. In: *Proc. Aceh Dev. Int. Conf.*; 2011:26–8.
4. The Insect Repellent DEET. Available at: Available from: http://www.epa.gov/pesticides/factsheets/chemicals/deet.htm. Accessed October 8, 2013.
5. Goddard J. Health risk and benefits of insect repellents. *[Internet]*. *Infect. Med.* 2002. Available at: Available from: http://www.medscape.com/viewarticle/438257_1. Accessed October 18, 2013.
6. Komisi Pestisida Departemen Pertanian. Metoda Standar Pengujian Efikasi Pestisida. 1995:4/9–95.
7. Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res*. 2005;19(4):303–309.
8. Departemen Kesehatan RI. *Farmakope Indonesia*. IV. Jakarta; 1995.
9. Mansjoer A. *Kapita selekta kedokteran*. jilid III. Jakarta: Media Aesculapius FKUI; 2000.
10. Jantan I, Zaki ZM. Development of environment-friendly insect repellents from the leaf oils of selected Malaysian plants. *ASEAN Rev. Biodivers. Environ. Conserv. (ARBEC [Internet]*.1–7. Available at: Available from: http://www.arbec.com.my/pdf/may-6.pdf.
11. Lukman A, Susanti E, Oktaviana R. Formulasi Gel Minyak Kulit Kayu Manis (Cinnamomum burmannii Bl) sebagai Sediaan Antinyamuk. *J. Penelit. Farm. Indones.* 2012;1(September):24–29.
12. G.C. Eka D, Hutagaol L. Pengaruh Penambahan Minyak Nilam (Patchouli oil) Sebagai Fiksatif Terhadap Stabilitas Aroma Parfum Melon Dan Maskulin Pada Saat Pemakaiannya. 2007:61.
13. Carter JS. *Dispensing For Pharmaceutical Student*. 12th ed. London: Pitman medical; 1975.
14. Martin AJS, Swarbrick, Cammarata A. *Farmasi fisika*. III. (Yoshita, ed.). Jakarta: Universitas Indonesia; 1993.
15. Ditjen POM DR. *Formularium Kosmetika Indonesia*. Jakarta: Departemen Kesehatan RI; 1985.
16. Voight R. *Buku Pelajaran Teknologi Farmasi*. IV. (Diterjemahkan oleh Soewandi NS, ed.). Yogyakarta: UI Press; 1995.
17. Ketaren S. *Pengantar Teknologi Minyak Atsiri*. Jakarta: Balai Pustaka; 1985.
18. Wasitaatmadja SM. *Penuntun Ilmu Kosmetik Medik*. Jakarta: UI-Press; 1997.
19. Lee J, Lee Y, Kim J, Yoon M, Choi YW. Formulation of Microemulsi Systems for Transdermal Delivery of Aceclofenac. *Arch Pharm Res*. 2005;28(9):1097–1102.

DIFFERENCES RESPONSE OF *Aedes aegypti,* L TO THE EXPOSURE OF AEROSOL INSECTICIDE CYPERPETHRIN AND EXTRACT OF TOBACCO LEAF (*Nicotiana tabacum*, LINN.)

Nur Endah Wahyuningsih, Devi Angelina Kusumaningtiar, Khairunnisa, Retno Hestiningsih
Faculty of Public Health, Diponegoro University
Correspondence: wahyuningsihnew@gmail.com

ABSTRACT

Background: Dengue hmorrhagic fever (DHF) is a disease caused by dengue virus and transmitted by *Aedes aegypti* mosquito. Control of the disease is mostly done with chemical insecticides. Chemical insecticides that are used continuously in the long term, lead to mosquitoes resistant, it is necessary to find an alternative control that does not cause resistance of using bioinsecticides. This study aimed to compare the results of exposure between control with aerosol insecticide with active ingredient cypermethrin and tobacco leaf on Lethal Concentration 50 (LC 50).
Method: This research uses the post test only control group design, Ae aegypti mosquitoes maintained in the laboratory of B2P2VRP Salatiga. A number of 720 mosquitoes exposed to insecticides, chemically and biologically. The mosquitoes that still alive after 24 hours holding, mated and wait until spawn. The eggs then were reared to be mosquitoes, and the rate of fecundity, fertility, larval pupal and mosquitoes survival are estimated. Analysis of the data using One Way ANOVA test, Kruskall Wallis and Mann-Whitney.
Result: The results showed to get LC50 of both types of insecticides, it is required 400 times levels of tobacco (10 %) compared with the levels of cypermethrin (0.025 %). The mosquito lifespan and fecundity rate are increased, different test result showed there were significant differences in fecundity rate on the tobacco exposure between LC 50 and control, p = 0.002 and of mosquito lifespan on cypermethrin exposure between control and LC50, p = 0.011 .
Keyword: fecundity, fertility, survival rates, Ae aegypti, cypermethrin insecticide, tobacco leaf

BACKGROUND

Aedes aegypti is the primary vector of dengue fever in tropical countries. Without a vaccine or other prevention of diseases, efforts to reduce cases of dengue fever and DHF limited to vector control. Vector control methods that most frequently used by the community is the application of insecticides.

Chemical insecticides are used continuously for long periods, causing mosquito resistance. Some insecticides had led resistance to insects, including organophosphate and organochlorine group of insecticides, and then the solution is created a new insecticide. The question is whether this new type of insecticide does not cause resistance to mosquitoes? Alternative vector control that does not cause resistance is the

use of natural insecticides. What about bio-insecticides from plants? Previous studies proved that insecticides plants do not lead to resistant mosquitoes.

Permethrin is a new class of insecticides commonly used to control insects that have been resistant to insecticides organophosphate and organochlorin class (Imperial Chemical Industries 1979). One of the active ingredients used in the formulation of aerosol insecticide is Cypermethrin. Cypermethrin is a pyrethroid class of WBA (water-based aerosols) type 2, which is more toxic than pyrethroids type 1. (Djojosumarto, 2008).

Three plants are known since a long time as a bio insecticide and has been produced commercially in several countries is Chrysanthemum cenerariaefolium (pyrethrin), Nicotiana tabacum (nicotine), and Derris spp. (Rotenone). Alkaloid nicotine content in tobacco leaves can be used as an insecticide. Alkaloids have a physiological effect, is an alkaline compound, bitter poisonous, containing carbon, hydrogen, nitrogen and oxygen in general has a cyclic form. Alkaloid compounds resulting from the metabolism of plants and is used as a backup for the synthesis of proteins. For plants, alkaloids serve as protection from pests, plant strengthening and regulating plant hormones.

Alkaloid nicotine, nicotine sulfate and other nicotine compounds used as contact poisons, fumigation, and stomach poison. This insecticide traded as Black Leaf 40 contains 40% nicotine, to control soft-bodied insects (Rangke, 1998). Nicotine readily soluble in water and ethanol to achieve success 95% (MOH, 1995) family of plants containing alkaloids are Liliaceae, Solanaceae and Papaveraceae (Tobias, 1998).

Aims of the study was to analyze mosquito fecundity, fertility and survival differences of Ae aegypti between mosquitoes exposed and not exposed to LC50 chemical aerosol insecticide with active ingredient of cypermethrin (0.0055 ppm/ 0.025%) and natural insecticides of tobacco leaves (Nicotiana tabacum, L, 0.0110 ppm/ 10%).

METHOD

The research was to analyze mosquito fecundity, fertility and survival differences Ae aegypti between mosquitoes exposed and not exposed to LC50 cypermethrin and tobacco leaf insecticides. Treatment of LC50 spraying with synthetic and bio insecticide will kill 50% of sample of mosquito. The treatment mosquitoes for this research are those that recover after holding 24 hours post spraying. Every cage had 15 couples (there were 30 mosquitoes consist of 15 males and 15 females) of Ae. aegypti with 6 replications. Mosquito using eggs that reared in B2P2VRP laboratory, Salatiga, Central Java. Development from egg until adult mosquito death, were followed. The results are used to compare the fecundity, fertility, larva survive, pupa survive and mosquito survive (life span) of Ae aegypti under a treatment and control design.

Two types of insecticides are used. It consists of insecticide aerosol cypermethrin and tobacco leaf as bio insecticide. LC 50 concentration for sipermetrin obtained after diluted to water to be concentration 0.0055 ppm (0.025%) with 2 x spray for each replication (6). Concentration for tobacco leaf is 0.0110 ppm (10%) with 3 x spray for each replication (6) using a modified Malaysian method. Exposure causes 50% Aedes death after holding for 24 hours. Treatment mosquitoes were Ae aegypti

that had been knocked down and that recovered after holding for 24 hours. The controls are mosquitoes that were not sprayed with insecticide. Analyses used were descriptive (%) and analytic using Anova, Post hoc test and Mann Whitney U. Dependent variables were fecundity, fertility rate (total egg hatches divided by total egg as a percentage), percent of larvae, pupae and mosquitoes that survived and mosquito life span compared between control and treatment.

RESULT

During the study, carried out measurements of temperature and relative humidity, and an average temperature of 25 º C, the maximum temperature of 26 º C and a minimum temperature of 24 º C. While an average relative humidity of 90%, maximum 96% and minimum 87%. Descriptive rates of fecundity, fertility, larva, pupa and mosquito survival can be seen in the following table.

Tabel 1. Descriptive of fecundity, fertility rates, larvae, pupae and mosquito survive between *Ae. aegypti* exposed and not exposed with Cypermethrin and tobacco leaf insecticides

			N	Mean	SD	Min	Max	Median
Fecundity rate	Cypermethrin	Control	6	28.9	4.5	22.1	34.1	30.6
		LC50	6	40.2	16.7	18.4	61.7	35.2
	Tobacco	Control	6	22.9	2.0	19.7	25.0	23.6
		LC50	6	14.3	3.1	11.3	18.6	13.5
Fertility rate	Cypermethrin	Control	6	84.7	6.0	77.3	93.8	85.1
		LC50	6	84.2	3.4	78.3	87.7	85.2
	Tobacco	Control	6	81.4	8.5	69.9	92.7	83.4
		LC50	6	74.5	10.7	55.0	83.1	77.6
Larvae survive	Cypermethrin	Control	6	53.9	3.2	48.3	57.1	54.6
		LC50	6	50.9	10.8	38.8	67.6	49.6
	Tobacco	Control	6	79.1	7.5	72.4	92.7	76.5
		LC50	6	74.2	8.1	63.6	83.8	73.8
Pupae survive	Cypermethrin	Control	6	99.1	0.9	97.7	100.0	99.0
		LC50	6	98.2	1.2	96.9	100.0	98.0
	Tobacco	Control	6	90.7	8.6	75.7	98.6	91.9
		LC50	6	84.3	8.3	75.4	98.6	81.4
Mosquito Survive (lifespan)	Cypermethrin	Control	6	26.0	1.8	23.0	28.0	26.5
		LC50	6	30.5	1.9	27.0	32.0	31.0
	Tobacco	Control	6	44.5	0.5	44.0	45.0	44.5
		LC50	6	44.3	1.0	43.0	45.0	45.0

The average total egg count for a couple with the Ae aegypti mosquito LC 50 treatment of cypermethrin was 40,2 and control 28,9 eggs, and this was higher and significantly different from the results for the Ae aegypti LC50 tobacco leaf 14,3 and

control 22,9 eggs. The fecundity rate differences was not significant for cypermethrin between LC50 and control p> 0.05, but the fecundity rate mosquito for tobacco between treatment and control was significantly different, p < 0.05. There were no significant difference in fertility, Larvae survive and pupae survive. Larvae survive is the percentage of larvae survival compared with total pupae survive is multiply 100%).

The mean larvae survival rate of the Ae aegypti mosquitoes LC50 treatment of cypermethrin was 50,9% and for control was 53,9% and this was not significantly lower than for the of tobacco leaf mosquitoes, LC50: 74,2 and control 79,1 %, the total average percent mosquito survival for larvae survival in cypermethrin between LC50 and control was not significantly different, p > 0.05, the same result is also for the total average percent mosquito survival for larvae survival in tobacco between LC50 and control was not significantly different, p > 0.05 (fig. 1).

The mean fertility and pupae rate of the Ae aegypti mosquitoes LC50 treatment of cypermethrin was 84,7 and 99,1 % and for control was 84,2 and 98,2 % and this was almost the same with tobacco leaf mosquitoes, LC50 : 74,5 and 84,3 % and control 81,4 and 90,7 %.

The average Ae aegypti mosquitoes surviveal of life span in the LC 50 cypermethrin treatment was 30,5 days and in the control it was 26,0 days. This was, lower compared to the average of Ae aegypti mosquitoes survival of life span in the LC50 tobacco leaf treatment of 45.0 and in the control of 44,5 days. The percent survival differences are significant for cypermethrin between LC50 and control p < 0.05, but the total average percent mosquito lifespan for tobacco between treatment and control was not significantly different, p > 0.05.

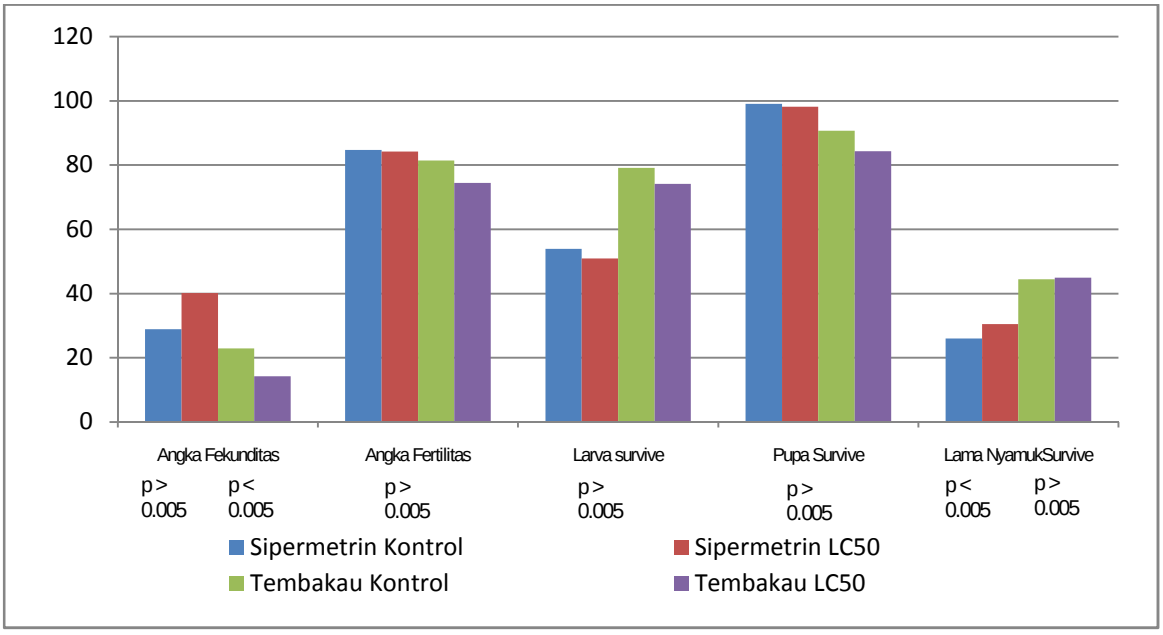

Figure 1. Fecundity, fertility rates, larvae, pupae and mosquito survive between *Ae. aegypti* exposed and not exposed with Cypermethrin and tobacco leaf nsecticides

Lifespan of mosquito

Average age of treatment mosquitoes survived for longer than the cypermethrin (LC50 32 days and control 28 days compared with tobacco mosquito 45 and 45 days, respectively, fig. 2. Test result show a significant difference of mean mosquito age between treatment and control for cypermethrin with $p < 0.05$ (fig. 1).

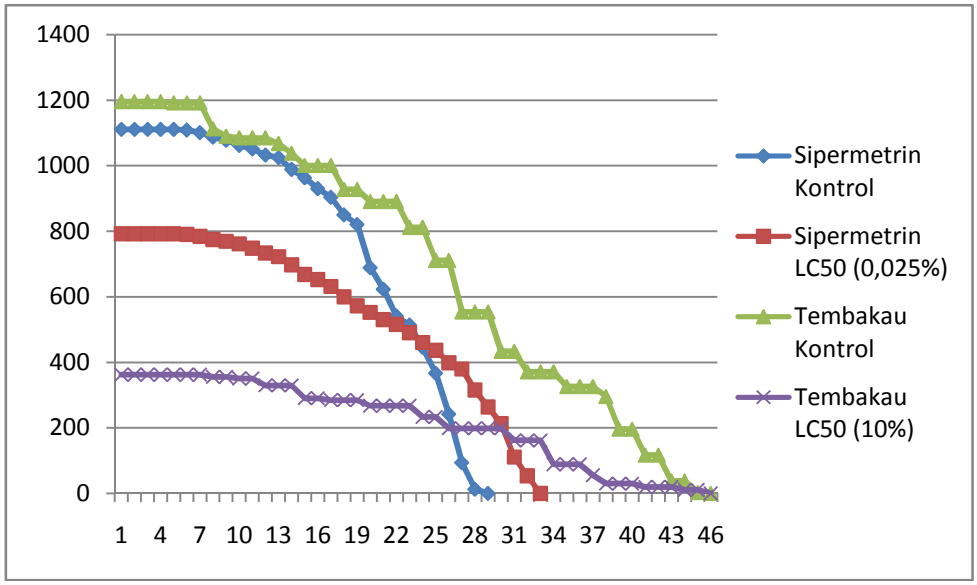

Figure 2. Life span of mosquito between *Ae. aegypti* exposed and not exposed with Cypermethrin and tobacco leaf insecticides

DISCUSSION

The average total egg count of Ae aegypti mosquito LC 50 treatment of cypermethrin was higher than control, it was the same with Wahyuningsih 2010 and 2007, that found fecundity rate of Ae aegypti was 43.7 and 38.4 eggs respectively.

Theoretically, female mosquitoes can lay eggs in batches of 100 - 200 at a time, but egg quantity depends on the female ability to consume blood and on the mosquito species (Russel & Jung, 1974). Egg hatches also depend on adaptation and temperature, and only hatch when in water (Russel & Jung, 1974).

The mean age of Ae. aegypti in treatment of Cypermethrin mosquitoes was 30.5 days with maximum of 32 days and for control it was 26 and 28 days respectively. The median age of mosquitoes with treatment of tobacco leaf was 45 days with a maximum of 45 days and for control it was 44.5 and 45 days respectively.

The mean age of Ae aegypti mosquitoes of Cypermenthrin showed a longer life span and significantly different, $p<0.05$, but tobacco leaf have median age longer than Cypermethrin treatment no significantly different.

This result different with Wahyuningsih 2009 that found chemical insecticide of propoxur cause longer lifespan than control (treatment of Ae aegypti for 76 days while control mosquitoes 18 days) and Wahyuningsih 2012 found insecticide of transfluthrin d allethrin cause longest lifespan than both bioinsecticide treatment and control

(mosquito treatment of transfluthrin d allethrin Ae aegypti for 36 days while bioinsecticide 29 days and control 22 days).

Result conclude to get LC50 of both types of insecticides, it is required 400 times levels of tobacco (10 %) compared with the levels of cypermethrin (0.025 %). The mosquito lifespan and fecundity rate are increased, different test result showed there were significant differences in fecundity rate on the tobacco exposure between LC 50 and control, p = 0.002 and of mosquito lifespan on cypermethrin exposure between control and LC50, p = 0.011.

REFFERENCE

Chareonviriyahpap T, Aum-aung B, Ratanatham S. Current insecticide resistance patterns in mosquito vectors in Thailand. Southeast Asian J Trop Med Public Health. 1999 Mar;30(1):184-94. http://www.ncbi.nlm.nih.gov/pubmed/?term=Chareonviriyaphap[author]+AND+Current+insecticide+resistance+patterns+in+mosquito+vectors+in+Thailand%2C+Southeast+Asian[Title], diakses 24 Nov 2013

Cui F, Raymond M, Qiao CL. Insecticide resistance in vector mosquitoes in China. Pest Manag Sci. 2006 Nov;62(11):1013-22. http://www.ncbi.nlm.nih.gov/pubmed/16953491], diakses 24 Nov 2013

DepkesRI,Farmakope Indonesia,Edisi IV,Jakarta: Departemen RI thn 95

Dyah, WS. Uji Ekstrak Daun Babadotan sebagai insektisida Nabati di laboratorium.Balaba.edisi 007 nomor 02 tahun IV

Djojosumarto. Pestisida dan Aplikasinya. hal. 109. Jakarta: Agro Media Pustaka. 2008.

Silva Hhg, Silva Ig, Lima Cg, Ovipositional Behavior of Aedes aegypti (Diptera, Culicidae) in Different Strata and Biological Cycle. 2003. 32 (1, 2, 3, 4): 1 – 8.

Sigit, S dan Hadi. Hama Permukiman Indonesia, Pengenalan Biologi dan Pengendalian, Unit Kajian Pengendalian Hama dan Permukiman. Bogor: Fakultas Kedokteran Hewan IPB, 2006

Imperial Chemical Industries, Permethrin-PH A New Broad Spectrum Insectiside for Public Health. hl-4,-- Rangke T. Kimia Bahan Alam. Jakarta: Departemen P dan K. Dir.Jen Pendidikan Tinggi. Proyek Pengembangan Lembaga Pendidikan Tenaga Kependidikan; 1998.1979.

Robert LL, Olson JK. Effects of sublethal dosages of insecticides on Culex quinquefasciatus. J Am Mosq Control Assoc. 1989 Jun;5(2):239-46. http://www.ncbi.nlm.nih.gov/pubmed/2746208, diakses 24 Nov 2013

Sawby R, Klowden MJ, Sjogren RD. 2013. Sublethal effects of larval methoprene exposure on adult mosquito longevity. J Am Mosq Control Assoc. 1992 Sep;8(3):290-2., http://www.ncbi.nlm.nih.gov/pubmed/1402867, diakses 24 Nov 2013

Selvi S, Endah MA, Nazni WA, Lee HL, Azahari AH, Resistance development and insecticide susceptibility in Culex quinquefasciatus against selection pressure of malathion and permethrin and its relationship to cross-resistance towards propoxur. Trop Biomed. 2005 Dec;22(2):103-13. http://www.ncbi.nlm.nih.gov/pubmed/16883275, diakses 24 Nov 2013

Tobing R.Kimia Bahan Alam.Jakarta: Departemen P dan K Dir.Jen Pendidikan Tinggi.Proyek Pengembangan Lembga Pendidikan Tenaga Kependidikan

Wudianto, R. Petunjuk Penggunaan Pestisida. Jakarta: Penebar Swadaya. 2008.

Wahyuningsih, N. E, Na'imah, 2007. Perbedaan Angka Fekunditas Dan Fertilitas Nyamuk Aedes Aegypti Pada Pemajanan Anti Nyamuk Aerosol, Bakar Dan Elektrik Berbahan Aktif D-Allethrin, 2007, (belum dipublikasi).

Wahyuningsih NE, Nurussakinah F and Siswoyowati. (2009). Aerosol propoxure insecticide impact towards Ae. aegypti and Ae. albopictus mosquito life times. Environmental Health International Seminar and Workshop, Research Paper Writing and Publication: Responding to Global Environmental Health Challenges, Indonesia, Bogor, Indonesia

Wahyuningsih NE dan Sihite RA, 2012. Pengaruh pemajanan bunga keluwih (Artocarpus camasi) dan anti nyamuk bakar berbahan transfluthrin dan d-alethrin terhadap angka fekunditas, fertilitas dan daya hidup nyamuk aedes aegypti, Kongres VIII dan Seminar Nasional Perhimpunan Entomologi Indonesia (PEI) tema: Peran & Tantangan Entomologi di Era Global, Bogor, 24-25 Januari 2012