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Vector Density And Resistance Status of Dengue Hemorrhagic Fever Mosquito (*Aedes aegypti*) Against Malathion in Manado City

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ABSTRACT

The success of DBD vector control depends on the vector susceptibility status of the insecticide used. Periodic monitoring of susceptibility to insecticide vulnerability is important. The purpose of this research is to know the vector density and the susceptibility status of Dengue Hemorrhagic Mosquito (*Ae.aegypti*) to the malation in Manado City. the benefits of this study are expected to provide information on the vector density and the susceptibility status of dengue fever mosquitoes (*Ae.aegypti*) to malathion in Manado City so that it becomes the evaluation material of DHF control program in planning in order to suppress the increase of DHF case in Manado City. This study is an observational descriptive study to obtain an overview of vector density and insecticide susceptibility status (malathion) in Manado city. The population in the study was the house and population of *Ae.aegypti* mosquitoes while the sample was a vector density of 100 houses. The results of the research The vector density is House Index (HI) = 25, Container Index (CI) = 12,57 and Breteau Index = 23,82 all show medium density of larvae and test results of *Ae.aegypti* mosquito susceptibility status using malathion 0, 8% impregnated paper and WHO standard susceptibility test, that *Ae.aegypti* mosquito susceptibility to malathion was done by counting the number of dead mosquitoes at one hour and 24 hours after exposure was stated to be resistant to malathion (mosquito mortality <80%). So immediately carry out the rotation of other insecticides by first testing the insecticides to be used.

INTRODUCTION

The entomology survey of DHF larvae development sites has often been carried out but it is not sustainable so that the presence of breeding sites is always available, which is supported by people's behavior by littering so that indirectly supports the existence of DHF vector mosquito breeding. So that in the implementation of supporting the DHF disease control program, the entomological survey is very important to predict vector density, which in turn can sharpen the implementation of DHF control programs in the area. Dengue Fever is widespread in various regions in Indonesia, especially in urban areas with the main vector is *Aedes aegypti* mosquitoes. There are 16 provinces that are reported frequently experiencing outbreaks of extraordinary event. The province with the most regency experiencing DHF outbreaks of extraordinary event is West Java. Other provinces that also often experience outbreaks of extraordinary event include: West Sumatra, South Sumatra, Bengkulu, Lampung, DKI Jakarta, Special Region of Yogyakarta, North Sulawesi, Central Sulawesi, South Sulawesi, Southeast Sulawesi, Bali, NTB, Maluku and Irian [1].

Manado City is one of the areas in North Sulawesi as DHF endemic area. Based on the Manado City Health Office report, every year there are cases of DHF occurrences and throughout 2017 there were 139 cases of dengue hemorrhagic fever cases of death in Kelurahan Malalayang 2. This case is spread to all of community Health centers in Manado. Minanga community Health center is the highest community Health center of DHF case throughout 2017 [2].

Considering that there are no medicines and vaccines to prevent dengue until now, the efforts to eradicate DHF are focused on eradicating mosquitoes in addition to early awareness of DHF cases. The control of DHF vector that has been done so far is controlling adult mosquitoes through fogging using malathion. Malathion is classified as an organophosphate insecticide that is often used by national programs to kill dengue vector mosquitoes by spraying in the form of mist (smoke) using a special machine (swingfog). *Aedes aegypti* which is the main vector of dengue fever, has been proven to be immune to various insecticides if contacted for a long time. This situation causes many problems in vector control programs in many countries. *Ae.aegypti* susceptibility study to insecticides in the western hemisphere found several cases of organophosphate resistance in Puerto Rico and in several countries in America [3]. Continuous application of insecticides on insects, especially *Ae. aegypti* can produce individuals who are tolerant and even immune to these insecticides. Research in Makassar shows a tendency to decrease *Ae aegypti* resistance against malathion in areas that are often applied with malathion [4]. The using of this malathion has long been applied in the city of Manado, so it can be estimated that there has been an increase in endurance of *Ae. aegypti* against malathion.

Insecticides when it used on a large scale, in a long period of time and high frequency can cause a decrease in susceptibility to target mosquitoes [5]. The emergence of resistant insect traits is triggered by long-term exposure. This occurs because the *Ae.aegypti* mosquito is able to develop an immune system against commonly used insecticides [6]. Success in controlling DHF vectors depends on the vector's susceptibility status to the insecticide used. Periodic monitoring of the vector's susceptibility status to the insecticide used very important. The data is as a basis and consideration for evaluating the using of insecticides further and to know the occurrence of resistance as early as possible [7].

Chemical vector control, especially the eradication of vectors using insecticides, which are used to eradicate mosquitoes will stimulate selection of the target insect population. Mosquitoes that are susceptible to the insecticide concerned will die, while the immune will remain alive. The amount of this life will gradually increase a lot, so that there is a development of mosquito immunity against the insecticide concerned. This immune event is one of the main inhibitors of chemical eradication of vectors with insecticides. This obstacle is felt to be very disruptive to the success of the business carried out, so it is necessary to do a test to find out the status of vector susceptibility to certain insecticides used for control. The purpose of this study was to determine the vector density and *Aedes aegypti* mosquito susceptibility status of malation in Manado City.

MATERIAL AND METHODS

This study was an observational descriptive study to get a picture of the susceptibility status of *Aedes aegypti* mosquitoes to malathion, the density of *Ae.aegypti* mosquito larvae carried out in 2018 in Malalayang 2 Village, Malalayang District, Manado City. The population in this study were all *Aedes aegypti* mosquito populations in Malalayang 2 Village, Manado City. The sample for testing the status of susceptibility to malathion is *Ae. aegypti* second generation (F2) results of colonization in the laboratory of the Environmental Health Department of Manado Health Polytechnic, Ministry of Health, obtained from the *Ae aegypti* mosquito larvae survey in Malalayang 2 Village Manado City. According to Fundacao National de Saude, the number of houses <60,000 uses 100 houses [3]. Determination of the sample by purposive sampling. The selection of houses for the larvae survey is based on the highest number of cases in Manado City which are placed in the DHF case house and around the case house (inside and outside the house). The instruments used were observation forms, larvae, resistance test tubes (test tubes and holding tubes), thermometers, hygrometers, mosquito cages and microscopes.

Work Procedures

1. Survey Implementation

The larvae survey was carried out at 100 houses both inside and outside the house. The results of the larvae survey were put into a sample bottle by labeling, then taken to the laboratory of the Environmental Health Department of Manado Health Polytechnic, Ministry of Health for colonization.

2. Colonization *Ae. aegypti* in the Laboratory

The *Aedes aegypti* colonization was conducted in the laboratory of the Environmental Health Department of Manado Health Polytechnic, Ministry of Health . The larvae from the survey results are then raised to adulthood by providing larvae / larvae feed.

3. Procedure testing for resistance (Malathion 0.8%).

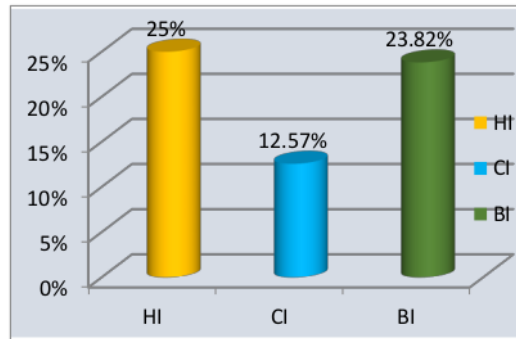
Susceptibility tests, 2 tubes for field mosquito testing and 2 tubes for mosquito control and in each test tube marked with red insecticide paper (0.8% malathion active ingredient WHO standard) in a circle. A total of 25 female mosquitoes were then put into a red marking test tube and exposed to malation insecticide for 1 hour. Mosquito female *Ae.aegypti* as a control of 25 tubes each was put into a tube that was marked green and equipped with paper without insecticide. Testing After 1 hour of exposure to insecticide then the mosquitoes are transferred into holding tubes marked with green markings, mosquito mortality is observed or counted after 24 hours of storage. The test is carried out twice in different times.

Analysis of vector density is done by calculating the percentage of House Index, Container Index and Breteau Index. The susceptibility of mosquitoes to malathion is done by counting the number of mosquitoes that die within one hour and 24 hours after exposure with an insecticide active ingredient malathion 0.8% (impregnated paper). If the death of a mosquito is > 98% then the mosquito is still considered vulnerable. Death 80% - 98% then the condition is tolerant. While mortality <80%, the mosquito is declared resistant.

RESULTS

1. DHF Vector Density in Malalayang 2 Village, Manado City

The DHF vector density survey in this study was carried out in 100 houses in Malalayang 2 Village, conducting a focused survey in Area 4 with consideration that the cases were the highest in the Kelurahan area. Based on the results of the average larval density survey HI = 25%, CI = 12.57%, BI = 23.82%. For more details, the graphic image below:



Based on the results of a survey of the density of DHF vectors in Malalayang 2 Village, Manado City can be analyzed as shown in the following graph:

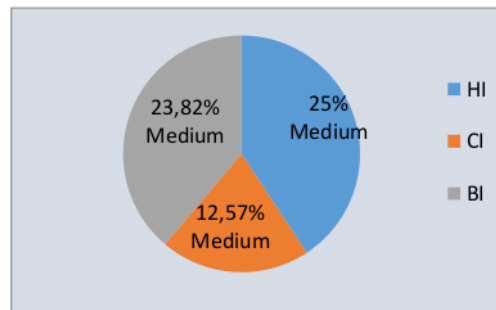


Figure 2. Density of larvae based on HI, CI and BI in Malalayang 2 village Manado City

2. Resistance Status of DHF Vector in Malalayang 2 Village

Susceptibility testing was carried out on adult mosquito samples from Malalayang 2 village using the susceptibility test kit, with WHO standard insecticide paper. This susceptibility test uses four test tubes and one control tube. Each tube contains 25 adult female *Ae aegypti* mosquitoes. The complete test results can be seen in table 2 below.

Table 2. Results of Adult Aedes aegypti Resistance Test Against Malation Insecticide (0.8%) WHO Standard in Malalayang Village 2 Manado City

Repeat	Treatment					Control				
	Observation 1 hour			Observation 24 hours		Observation 1 hour			Observation 24 hours	
	Number of Mosquitoes that were tested	Number of dead	% Dead	Number of dead	% Dead	Number of Mosquitoes that were tested	Number of dead	% Dead	Number of dead	% Dead
I	25	0	0	3	12	25	0	0	0	0
II	25	0	0	0	0					
III	25	0	0	2	8					
IV	25	0	0	2	8					
	Average	0	0	1,75	7	Average	0	0	0	0
Temperature	27-29°C			27-29°C		27-29°C			27-29°C	
humidity	70-80%			72-80%		75-80%			75-80%	

DISCUSSIONS

The results of a survey conducted on several DHF vector indicators in Malalayang 2 Village showed that the House Index (HI) value was 25%. This figure does not meet the national standard of <5% for HI. The CI and BI indicators show an average of 12.57% and 23.82. These figures if adjusted to the Density figure table are included in the category of medium density because they are at level 5. The results of this study are higher in Utan Kayu Utara Village, East Jakarta where found HI 11.5%, CI 6.5 % and BI 13.3, which means that the density of DHF vectors in Malalayang 2 Village of Manado City has a higher risk of dengue virus transmission or transmission of dengue disease than in North Utan Kayu, East Jakarta [8].

If an area has HI more than 5%, it indicates that the area has a high risk for transmission of dengue, if HI <5%, then prevention can be done for dengue virus infection. It was further explained that if HI > 15% means the area has already had a case of DHF. the higher the HI number, means the higher the density of mosquitoes, the higher the risk of people in the area to contact with mosquitoes and also to be infected with dengue virus. Case data in Malalayang 2 Village throughout 2017 32 cases of DHF occurred, which is very likely closely related to high HI, with found 25% of houses have larvae vector DHF.

The high density of larvae at the study site in Malalayang 2 village in Manado City was influenced by the community's lack of participation in eradicating mosquito nests such as closing water reservoirs, draining and scrubbing the tub / toilet properly and correctly, thus causing mosquito eggs Aedes sp can hatch into larvae. states that the most effective DHF prevention and prevention efforts that can be carried out by individuals, families and communities is by eradicating the infectious mosquitoes, especially their larvae, through the eradication of mosquito nests.

The results of this study also showed that the type of breeding place most loved by mosquitoes to lay eggs is a bucket. This information gives a hint to the people in Malalayang 2 Village that the draining and cleaning of the bucket must be carried out every 3 days to remove the mosquito eggs that are sticking, so that it does not become a breeding ground for Aedes sp mosquitoes in a bucket that has a large / wide container and is not closed provide easy access for adult mosquitoes to lay eggs., containers that tend not to be closed must be accompanied by draining and brushing activities, when compared to containers that can be closed like a bucket that is easier to control mosquitoes Aedes aegypti for laying eggs

Resistance test results showed that Ae. aegypti mosquitoes that died after exposure with 0.8% malathion were 7% of the samples tested, these results showed that Ae.aegypti mosquitoes in Malalayang 2 village were already resistant. Decreased resistance status of Ae aegypti mosquito population in Malalayang 2 village is very possible because of the use of malathion insecticides in the control of DHF through fogging programs both from the Community Health Center and the Manado City Health Office. The condition of the study area that was exposed to malathion insecticide for DHF control program has triggered the resistance of Ae aegypti mosquitoes in the region. The use of malathion massively and for a long time since 1970 in Jakarta and since 1975 in Palu by the relevant agencies (Health Office and Community Health Center) in the dengue case control program has contributed to the resistance. Previous studies reported Ae aegypti mosquitoes has been resistant to malathion insecticides [9].

Another factor that is likely to contribute to triggering mosquito resistance in Malalayang 2 Village is the use of household insecticides by the local community. The results of interviews with the community show that the average

community uses household insecticides in their efforts to avoid mosquito bites. Selective pressure from household insecticides (pyrethroids) helps increase the non-specific esterase enzyme of the mosquito population, which in turn will accelerate mosquito immunity with these insecticides [7].

The using of larvicides as an effort to control mosquitoes *Ae. aegypti* at the larval stage distributed by Community Health Center staff may contribute to resistance in the mosquito population in Malalayang 2 Village. Research results in Jakarta indicate that *Ae. aegypti* larvae has been resistant to the use of temefos [10].

In addition to these factors there are other factors that are very influential on increasing the resistance status of a mosquito population, namely the nature of the mosquito itself. Supporting mosquito traits are mosquito populations that are dynamic, adaptable and have a high evolution. Mosquitoes also have a high reproductive speed and have a short generation period so that mosquitoes easily undergo genetic mutations. The more the number of generations every year, the speed of resistance will be faster [11]. This is evidenced by the research report which shows that changes in resistance level *Ae. aegypti* became 4.25 times in only eight generations of malation [12].

The results of measurements of high larvae density and the nature of *Ae. aegypti* mosquitoes that are already resistant to malation 0.8% in Malalayang 2 Village are a sign of caution both from related institutions and the local community, because this condition can cause a high incidence of DHF cases and cannot controlled quickly by using an insecticide that is commonly used, namely malation. This condition requires the use of substitute insecticides (rotation insecticides), preferably using insecticides from different groups, to control dengue vectors, in addition to implementing a program to eradicate mosquito nests in the community continues to be implemented, as the most effective DHF vector control efforts.

CONCLUSION

Larvae density measured from the three parameters, namely **House Index (HI) = 25.0**, **Container Index (CI) = 12.57** and **Breteau Index (BI) = 23.82** all show medium larvae density. *Ae. aegypti* mosquito in Malalayang 2 Village, Manado City, is already resistant to 0.8% malathion insecticide.

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