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Detection of Insecticide Resistance in *Aedes aegypti* to Organophosphate in Pulogadung, East Jakarta

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Abstract

Dengue Hemorrhagic Fever (DHF) is a major public health problem in Indonesia. Jakarta is a capital city with the highest number of dengue patients. Among sporadic endemic areas in Jakarta, Pulogadung, a district of East Jakarta, is one of the endemic areas of this disease. The primary strategy for the control of DHF is based on reducing population densities of the main mosquito vector *Aedes aegypti*. Organophosphate is an insecticide that has been used for more than 25 years in dengue vector control program. The long term used and sublethal dosage of this insecticide can induce resistance. This laboratory study used microplate test and ELISA reader to determine the increase of alfa- esterase activity in *A. aegypti* larvae for detecting the resistance to organophosphate. Resistance pattern of *A. aegypti* to organophosphate insecticide in RW 01 Pulogadung was shown to be: 23% high resistant, 33% medium resistant and 44% sensitive. This result was highly related to local community behavior where we found that the use of insecticide spray by the people was very low (8.8% of the sample). We found that the people who used insecticide spray were only 8.8% of the sample. Therefore, organophosphate still can be used in this area to control the DHF in the future. Based on resistance pattern of *A. aegypti* to organophosphate insecticide in *Rukun Warga* (RW) 01 Pulogadung, we can conclude that organophosphate still can be used in this area to control the DHF in the future.

Abstrak

Deteksi Resistensi Aedes Aesgypti terhadap Insektisida Organofosfat di Pulogadung Jakarta Timur. Demam Berdarah Dengue (DBD) merupakan masalah kesehatan masyarakat di Indonesia. DKI Jakarta merupakan propinsi dengan jumlah penderita DBD terbanyak. Pulo Gadung Jakarta Timur merupakan salah satu daerah endemis DBD dan beberapa wilayah lainnya di DKI merupakan daerah sporadis penyakit tersebut. Strategi pengendalian utama DBD masih ditekankan pada pemberantasan vektornya yaitu *Aedes aegypti* (*A. aegypti*). Sampai saat ini insektisida golongan organofosfat adalah insektisida yang telah digunakan lebih dari 25 tahun untuk pengendalian vektor DBD. Penggunaan insektisida tersebut dalam waktu lama dan dosis subletal dapat menginduksi terjadinya resistensi. Pada penelitian ini dilakukan uji *microplate* dengan ELISA *reader* untuk mengetahui resistensi serangga terhadap organofosfat. Resistensi diketahui dengan adanya peningkatan aktivitas enzim esterase non spesifik. Pola resistensi *A. aegypti* terhadap organofosfat di RW 01 Pulogadung menunjukkan hasil sebagai berikut: 23% sangat resisten, 33% resistensi sedang dan 44% sensitif. Hasil ini berkaitan erat dengan rendahnya frekuensi penggunaan obat nyamuk semprot oleh masyarakat (8,8% sampel). Berdasarkan pola resistensi *A. aegypti* terhadap organofosfat di wilayah Rukun Warga (RW) 01 Pulogadung, kami menyimpulkan bahwa organofosfat masih dapat dipakai dalam pengendalian DBD di wilayah tersebut.

Keywords: Aedes aegypti, insecticide, larvae, resistance, organophosphate

Introduction

Dengue Hemorrhagic Fever (DHF) is a viral disease that spreads rapidly. According to WHO, 2009 its incidence rate has increased 30-time within the last 50 years and in the last decade it has spread from urban to a rural area.[1] Based on the data of Ministry of Health, in DHF Bulletin, 2010[2] in Indonesia DHF has been first identified in Surabaya and Jakarta in 1968, and has spread to 32 provinces (97%) and 382 districts/cities (77%) in 2009. Although there was a decrease in the incidence rate of this disease nationally, Jakarta held the highest record during 2004-2009 and its occurrence was believed to be caused by the effect of its population density, high mobility, and better transportation facilities than other regions.

Dengue Hemorrhagic Fever is transmitted by *Aedes aegypti* (*A. aegypti*) mosquito as the actual vector and *Aedes albopictus* as the potential vector. Until now, there is no specific drug that has been used for treatment and the prevention vaccine has not been found yet. Therefore, the primary strategy for control of DHF is based on reducing population densities of the main mosquito vector. According to WHO guidelines,[1] chemical insecticides that are used to eliminate larvae and control the adult mosquitoes are from the groups of organo-phosphates and pyrethroids. These insecticides are not only used during the outbreak but also widely used by the community especially in big cities even when there is no outbreak. Various types and brands use organophosphate or carbamate as an active ingredient. Unknowingly this condition becomes a contributing factor to the development of insecticide resistance. Exposure to this active ingredient will increase the non-specific esterase enzymes in the mosquito, and it can affect the susceptibility of the mosquito to those insecticides. The increase of this enzyme can be detected by an enzymatic reaction. Meanwhile, the degree of mosquitoes' susceptibility can be measured by ELISA test to see the Absorbance value.

Based on the data from Jakarta Provincial Health Office (Suku Dinas Kesehatan), an organophosphate insecticide that is used to control the adult mosquito by fogging in Jakarta is Malathion and Temephos, which is larvacides that have also been used to kill the stadium larvae of *A. aegypti*. Malathion has been used massively by the government since 1969 and Temephos have been widely used since 1980 in Dengue eradication program. The long-term use of these insecticides can cause resistance to their active ingredient. Studies of *A. aegypti* resistance to this organophosphates that focused on 2 areas such as Tanjung Priok, North Jakarta and Mampang Prapatan in South Jakarta was conducted by Zulhasril and Suri, 2010.[3] The result of the studies in both areas showed that resistance of DHF vector to this organophosphate groups had occurred. The aimed of this study is to know the distribution and condition of the DHF vector resistance to this insecticides through biochemical assay by detecting the increase of non-specific esterase enzyme in another part of Jakarta and to complete and map the resistance in Jakarta.

Methods

The location of the study was conducted in Kecamatan Pulogadung where, based on data from *Suku Dinas Kesehatan DKI Jakarta*, the incidence of DHF was high. The sampling locations were randomly selected to choose one *Rukun Warga* (RW) and then 8 *Rukun Tetangga* (RT) in that RW, and 5-10 houses from each selected RT. *Aedes aegypti* eggs collection was done by setting an ovitrap. 2 ovitraps were put in a place which was not exposed to direct sun and hidden inside the selected house so the mosquitoes will not be disturbed by human activities in that house. In this study, we also interviewed the owner or people of the randomly selected house in the form of a questionnaire concerning the daily use of insecticide. The study took place between July-November 2013.

In the laboratory, *A. aegypti* eggs collected from the field were immersed in enamel pan measuring 20 cm x 40cm x 5cm. The larvae which have hatched were transferred to another container and then given food. Once every 2 days, the water in that enamel pan will be replaced by fresh water until larvae grow into stage IV, ready to be used in experiments for a resistance test. Some larvae that were reared into adult mosquitoes will be put into a cage measuring 50 cm^2 and fed with the blood of guinea pigs. The eggs of these mosquitoes were kept as a stocks for other experiments. Larvae obtained from collections in a variety of containers at the sampling site were directly used.[4]

The activity of esterase enzyme was measured according to the method of Lee.[4] *A. aegypti* larvae were pipetted and put into wells on the ELISA plate, one larva in each well. Excess water was dried using filter paper. After it is dried, the larvae will be finely ground using a mortar and then homogenized by adding 0.02 M PBS solution at pH 7.0. The homogenate was transferred to Becton Dickinson ELISA plate which was placed on ice. Five larvae were taken from each RT, and each mosquito larva homogenate was made into 4 replications. Then substrate solution (composed of α - naphthyl acetate in acetone (6 g/L) plus PBS solution 0.02 M at pH 7.0) was added to each well, and the enzymatic reaction would take place. After that, another *coupling reagent* solution (a mixture of fast blue B salt + distilled water and sodium dodecyl sulfate 5%) was added to each well (let it stand for 1 minute) until the color of the reaction mixture changed from red (pink) to blue. This biochemical reaction was stopped by adding 10% acetic acid solution into each well. Color intensity of the resulting reaction was read using ELISA Reader at 450 nm using bidets water as a control.[5] OD (optical density) was calculated for each RT. (Results were classified into sensitivity categories using Lee's criteria as in Table 1. Proportion statistical test was performed using SPSS 19).

Table 1. Sensitivity Class by Optical Density Values

OD Value	Sensitivity Class
0.0-0.7	Highly Sensitive
0.7-0.9	Moderately Resistance
> 0.9	Highly Resistance

Remarks: OD= Optical Density

Results and Discussion

Aedes aegypti eggs collected from the field were reared in the laboratory, and they produced larvae with a number between 74 and 443 as can be seen in Table 2. A large number of larvae is likely due to the condition of the dense residential area with houses that are adjacent to one another, causing lack of lighting inside the house. A large number of larvae found at RW 01 is a factor that might contribute to a high prevalence of Dengue Hemorrhagic Fever in this region, though there was a decrease in cases in 2013 when compared to the previous year.

Principally the resistance mechanisms would prevent insecticide to bind to its target binding site such as acetylcholinesterase or by increasing enzyme activity such as esterases to detoxify active ingredients before reaching the target point. Factor that affects the mechanisms of insecticide resistance in *A. aegypti* is genetic. This factor depends on the presence of resistance genes that are capable of coding certain enzymes formation in the mosquito to neutralize the existence of such insecticides, e.g., acetylcholine esterase enzymeb, Melo-Santos *et al*.[6] In this study, the resistance of *A aegypti* to organophosphate insecticides was seen through the increase of esterase enzymes. The reaction of esterase with naphthyl acetate substrate produces naphtol which can be seen by the color changes. The increase of α-esterase enzyme was indicated by the formation of blue, green to dark blue color.

Various studies showed that resistance of *A. aegypti* to organophosphates has been found in several countries, such as the studies were done by Aziz *et al*.[7] in Makkah, Saudi Arabia; Polson *et al*.[8] in Trinidad and Tobago; Shafie *et al*.[9] in urban areas in Shah Alam and Pantai Dalam In Malaysia. In Jakarta, Zulhasril and Suri's research,[4] has also reported the resistance in *A. aegypti* in Tanjung Priok (North Jakarta) and Mampang (South Jakarta). The results of *A. aegypti* non-specific enzyme resistance to organophosphate with ELISA can be seen in Table 3. The result showed 23% of the larvae are highly resistance; 33% are moderately resistance to organophosphate, and 44% are highly sensitive as seen in Figure 1.

Even though the results of this study showed a relatively low percentage of high resistance (RR) *i* which is only 23%, the amount of moderate resistance (RS) reached up to 33%. Therefore, precaution is needed so that the moderate resistance larvae will not develop into high resistance larvae. Thus, the monitoring of insecticides resistance is required periodically. This is consistent with a study that has been done by Melo-Santos *et al*.[6] in some areas in Brazil and in 10 regions in Colombia by Ocampo *et al*.[10] which stated that the surveillance program for insecticide resistance needs to be done. The resistance pattern of *A. aegypti* to organophosphate insecticide in RW 01 Pulo Gadung led to the assumption that this organophosphate insecticide groups can still be used in that area to control DHF.

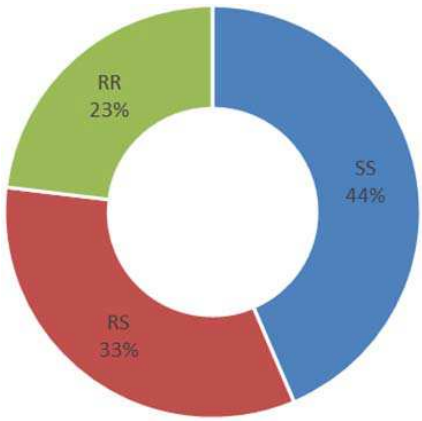
Research was done by Shetty *et al*.[11] in Karnataka, India and study by Karunaratne[12] in Sri Lanka showed a gradation of *A. aegypti* resistance. The studies showed the presence of moderate levels of resistance to organophosphates although most of the research areas have been very resistant to organophosphates. The same result was found in our study at RW 01 Pulo Gadung region, where 33% of larvae showed moderate levels of resistance and 44% are still very sensitive.

Table 2. Number of Larva Collected

RT	Number of Larvae
1	205
2	443
3	273
5	271
7	114
9	394
10	414
11	74

Table 3. Sensitivity of Larvae from each RT

RT	Numbers of larvae		
	Highly sensitive	Moderately resistance	Highly resistance
1	3	2	0
2	3	2	0
3	4	1	0
5	0	3	2
7	3	2	0
9	0	0	5
10	1	2	2
11	3	1	0



SS= Very Sensitive; RS= Moderate Resistance; RR=High Resistance

Figure 1. Percentage of Vector Resistance

Table 4. Percentage of Resistant Larvae

RT	%S	%R
1	60	40
2	60	40
3	80	20
5	0	100*
7	60	40
9	0	100*
10	20	80*
11	75	25

The big difference in the results of research in RW 01 Pulo Gadung with some studies earlier is possibly related to the habit of the people who live in that region.

We found that the use of insect spray by the people was very low (8.8% of the sample). This causes less exposure of insecticide to mosquito, so the development of resistance process is running very slow. The percentage of resistance level in 8 (eight) RT was calculated by dividing the amount of sensitive/resistance larvae with total larvae that were examined. The resistance level of eight RT ranged from 20% to 100% as seen in Table 4.

The pairwise proportional test showed that the level of resistance is separated into 2 groups which are statistically significant. For example, a group of RT 1, 2, 3, 7, and 11 have a resistance level of 20-40%, and the other group consisting of RT 5, 9, and 10 have a resistance level of 80-100 %. The resistance level among RT within each group is not statistically different.

Conclusions

The pattern of *A. aegypti* resistance to organophosphates in RW 01 Pulo Gadung showed a gradation of resistance. Precaution is needed so that the moderate resistance larvae will not develop into highly resistance larvae. The results of this data can be used as a basic data to make policy in DHF vector control programs especially in Jakarta. Periodically monitoring of the mosquitoes' susceptibility to the extensive widely used insecticides is necessary to ensure that an appropriate choice of chemicals is used to make the eradication program of DHF successful.

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