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Resistance Status of Dengue Virus Serotype in Aedes Aegypti on The Exposure of Insecticide Temefos and Cypermethrin

Arimaswati[1]; La Ode Alifariki[2]; Heriviyatno Julika Siagian[3*)]; Tukatman[3]; La Rangki[4]

[1]Medical College, Halu Oleo University, Kendari
[2]Departement of Epidemiology, Halu Oleo University, Kendari
[3*)]Departement of Nursing, Sembilanbelas November University, Kolaka
[4]Departement of Nursing, Halu Oleo University, Kendari

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ABSTRACT

The success of vector control is influenced by the resistance of Ae. aegypti against insecticides. The continuation use of insecticides will leave a resistant population. The study aims to determine resistance status of dengue virus serotype in Aedes Aegypti on the exposure of Insecticide Temefos and cypermethrin. Mosquitoes are taken directly from the tub and the home environment using the GAMA DOTIK. To determine the status of resistance to organophosphate insecticides in larvae and synthetic pyrethroids in adult mosquitoes, a biological test was carried out using the WHO 1992 method at a diagnostic dose (temefos 0.02 ppm and 0.05% cypermethrin), while the serotype of the dengue virus was determined by the Reverse Transcriptase method. -Polymerase Chain Reaction (RT-PCR) using Lanciotti-specific primers. Endemic areas (Gadingan, Wonosidi Lor and Dipan) are all still susceptible to temefos with 100% larval mortality. Sporadic areas such as Driyan were in a vulnerable status with Mortality Rate (AK) = 100%, Durungan and Kriyanan were in a tolerant status (AK = 88% and 97%). Potential areas such as Kauman and Janten were vulnerable (AK = 100% and 98%), Panjatan was in tolerant status (AK = 84%). Endemic, sporadic and potential areas were all resistant with AK = <80% to the insecticide cypermethrin. Dengue virus serotypes in endemic areas are dengue 2 and dengue 3. In potential areas, dengue serotype 3 is found, while in sporadic areas there are no virus serotypes. Conclusion: The resistance status of Aedes aegypti in endemic, sporadic and potential areas to organophosphate insecticides (temefos) is in the susceptible to tolerant range, while the all areas status to synthetic pyrethroids (cypermethrin) are resistant.

Status Resistensi Virus Dengue Nyamuk Aedes Aegypti pada Paparan Insektisida Temefos dan Cypermethrin

Kata kunci:

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Cypermethrin
Temefos

**) corresponding author*

Departement of Nursing, University of Sembilanbelas November, Kolaka

Email: heriviyatno.j.siagian@gmail.com

ABSTRAK

Keberhasilan pengendalian vektor dipengaruhi oleh hambatan Ae. aegypti melawan insektisida. Penggunaan insektisida secara terus menerus akan meninggalkan populasi yang resisten. Penelitian ini bertujuan untuk mengetahui status resistensi serotipe virus dengue Aedes Aegypti terhadap paparan Insektisida Temefos dan cypermethrin. Nyamuk diambil langsung dari bak mandi dan lingkungan rumah menggunakan GAMA DOTIK. Untuk mengetahui status resistensi insektisida organofosfat pada larva dan piretroid sintetik pada nyamuk dewasa, dilakukan uji biologis dengan metode WHO 1992 dengan dosis diagnostik (temefos 0,02 ppm dan 0,05% cypermethrin), sedangkan serotipe virus dengue ditentukan dengan metode Reverse Transcriptase. -Polymerase Chain Reaction (RT-PCR) menggunakan primer khusus Lanciotti. Daerah endemik (Gadingan,

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Wonosidi Lor dan Dipan) semuanya masih rentan terhadap temefos dengan kematian larva 100%. Daerah sporadis seperti Driyan berada dalam status rawan dengan Angka Kematian (AK) = 100%, Durungan dan Kriyanan dalam status toleran (AK = 88% dan 97%). Wilayah potensial seperti Kauman dan Janten termasuk kategori rawan (AK = 100% dan 98%), Panjatan dalam status toleran (AK = 84%). Daerah endemik, sporadis dan potensial semuanya resisten dengan AK = <80% terhadap insektisida cypermethrin. Serotipe virus Dengue di daerah endemis adalah Dengue 2 dan Dengue 3. Di daerah potensial ditemukan Dengue Serotipe 3, sedangkan di daerah sporadis tidak terdapat serotipe virus. Status resistensi nyamuk Aedes aegypti di daerah endemik, sporadis dan potensial terhadap insektisida organofosfat (temefos) berada pada rentang toleran, sedangkan semua status daerah terhadap piretroid sintetis (cypermethrin) resisten.

Introduction

Dengue Hemorrhagic Fever (DHF) is one of the health problems caused by the dengue virus and is transmitted by the mosquito vectors Aedes aegypti and Aedes albopictus (Alifariki & Mubarak, 2017). Indonesia is in the first place of dengue cases in Southeast Asia. The Ministry of Health reported 112,511 DHF cases in 2013 with a death rate of 871 people (Kemenkes, 2013). The Yogyakarta Provincial Health Office (Dinkes DIY) recorded a significant increase in cases in 2010 with a total of 5,121 cases where the number of deaths was 33 cases (CFR 0.64%) and the mortality rate decreased in 2011 to 0.5% (Kemenkes, 2015).

The number of DHF cases in the Wates community health center work area is the highest in the area with a total of 10 cases. In addition, there is an increase in endemic villages to 43 villages and potential villages to 3 villages compared to the previous year, namely 37 endemic villages and potential villages (Kulon Progo District Health Office, 2020).

The John Gordon's triangle theory states that the dengue virus is the cause of DHF. Dengue virus (virDEN) with 4 serotypes such as DEN-1, DEN-2, DEN-3, DEN-4 is able to circulate throughout the year. The dengue virus lives in the body of the mosquito, making mosquitoes virulent for life (Harapan et al., 2019),(Rodenhuis-Zybert et al., 2010). Data on the proportion of mosquitoes infected with the dengue virus are important for dengue eradication planning, especially those related to vector control (Boesri & Susanti, n.d.). Serotype of the dengue virus originating from the Ae Aegypti mosquito in Kulon Progo Regency has never been reported.

Vector control aims to break the chain of transmission. The success of vector control is influenced by the resistance of Ae. aegypti against insecticides. The continuation use of insecticides will leave a resistant population. Ae aegypti Mosquito is able to maintain and pass on resistance traits to their offspring for a long time (Untung, 2005). The Kulon Progo Regency Government carried out the Eradication of Mosquito Nests (PSN) using organophosphate and synthetic pyrethroid insecticides through fogging resting (Kulon Progo District Health Office, 2020). Indriani stated that the intervention used organophosphates at breeding sites for Ae aegypti mosquitoes can reduce transmission (Indriani, 2002). Resistance tests and evaluation of insecticide used have never been reported in Kulon Progo District. Therefore, it is necessary to conduct research on the status of resistance to synthetic organophosphate (Cypermethrin) and pyrethroid insecticides (Temefos) and to determine the serotype of dengue virus in Ae.aegypti mosquitoes in Kulon Progo Regency.

Method

The biological resistance test of Ae.aegypti against temephos and cypermethrin is a quasi experimental study with the design of the post only control group design. Dengue virus serotype determination was an observational-analytic study with a case-control design.

Sample

The sample used was the Ae. aegypti female F1 larvae and adult phases colonized in the Parasitology Laboratory of the Faculty of Medicine UGM, which came from F0 larvae taken from endemic, sporadic and potential areas in Kulon Progo Regency. Each area is represented by 3 sampling locations. For the bio-test with temefos, the mosquitoes used were Ae aegypti, instar 3 larvae phase, while female adult mosquitoes aged 3-5 days were used for biological tests with cypermethrin. To determine the serotype of the dengue virus using adult female mosquitoes aged more than 7 days.

Resistance status

The WHO standard method is used to determine insecticide resistance status. Biological resistance test for Ae.aegypti against temefos use a diagnostic dose of 0.02 ppm. Biological testing was carried out in 4 treatment groups with 1 control group. Each group contains 25 test larvae. The biological resistance test of adult mosquitoes to cypermethrin used a dose of 0.05% in impregnated paper. Biological testing was carried out on 3 treatment groups and 1 control group. Each treatment contains 10 test mosquitoes.

Dengue virus serotype determination

Dengue virus serotype was determined by RT-PCR method using Roche TITAN One Tube Kit (Cat No. 11 939 823 001). RNA extraction used 10 mosquitoes (for each sampling location) which had been separated from the thorax. RNA extraction products were stored at -800C if not used immediately. CDNA formation used one step RT-PCR, and DNA amplification using nested RT-PCR.

Data analysis

Observations were made for 1 hour to see the knocked down mosquitoes and continued 24 hours to see the percentage of mosquito mortality calculated according to Davidson and Zahar's criteria, namely: vulnerable if the percentage of mortality is ≥98%, tolerant = 80– <98%, resistant = <80% (Macoris et al., 2007). Dengue virus serotype was analyzed by electrophoresis on 2% agarose gel using a specific primer from Lanciotti

Results and Discussion

Ae.aegypti Larvae resistance Test against Organophosphate (temefos)

Results of biological resistance test for larvae *Ae. aegypti* against larvicide temephos with a diagnostic dose of 0.02 ppm in endemic, sporadic and potential dengue areas in Kulon Progo Regency is presented in Table 1.

Table 1 shows the mortality of *Ae. aegypti* larvae after being exposed to temefos 0.02 ppm for 24 hours in each area in Kulon Progo Regency. Based on the criteria of Davidson and Zahart in 1973, *Ae.aegypti* in endemic areas (Gadingan, Wonosidi Lor, Dipan) are all vulnerable (mortality 100%). For sporadic areas, the Driyan area was vulnerable (100%), while the Durungan and Kriyanan areas were classified as tolerant (88% and 97% mortality). In potential areas, Kanoman and Janten are vulnerable (100% and 98%), while Panjatan is classified as tolerant (84% mortality).

Table 1
The number and percentage of Ae. aegypti mortality after exposure with 0.02 ppm temefos larvicide

Location	Number of mosquito samples	Mortality (%)	Status
Endemic area			
Gadingan	100	100	Vulnerable
Wonosidi Lor	100	100	Vulnerable
Dipan	100	100	Vulnerable
Sporadic area			
Durungan	100	88	Tolerant
Kriyanan	100	97	Tolerant
Driyan	100	100	Tolerant
Potensial area			
Janten	100	98	Vulnerable
Kanoman	100	100	Vulnerable
Panjatan	100	84	Tolerant
Control	100	0	-

Ae. aegypti Adult resistance test against Synthetic Pyrethroids (cypermetrin)

The results of the biological resistance test of adult Ae aegypti mosquitoes to cypermethrin at a dose of 0.05% from endemic, sporadic and potential areas in Kulon Progo Regency are presented in Table 2.

Table 2 shows the Ae. aegypti from endemic, sporadic and potential areas were exposed to 0.05% cypermethrin for 60 minutes and 24 hours to calculate the percentage of mortality. Based on the criteria of Davidson and Zahar in 1973, all Ae aegypti mosquitoes in endemic, sporadic and potential areas were resistant with a mortality rate <80%.

Tabel 2
Number and percentage of adult Ae Aegypti mosquitoes after exposure to 0.05% cypermethrin

Location	Number of mosquito samples	Mortality (%)		Status
		60 minutes	24 hour	
Endemic area				
Gadingan	30	20	0	Resistant
Wonosidi Lor	30	20	6,67	Resistant
Dipan	30	26,67	6,67	Resistant
Sporadic area				
Durungan	30	26,67	10	Resistant
Kriyanan	30	30	30	Resistant
Driyan	30	6,67	0	Resistant
Potential area				
Janten	30	13,33	10	Resistant
Kanoman	30	20	13,33	Resistant
Panjatan	30	16,67	13,33	Resistant
Control	10	0	0	-

Dengue Virus Serotype Determination

The RT-PCR method used in this study was to detect dengue virus serotypes in *Ae. aegypti* based on the size of the product produced. The amount of PCR product produced was in accordance with the band size on the 100bp DNA marker, *ladder* namely 482bp for dengue 1, 290bp for dengue 2, 119 for dengue 3 and 389bp for dengue 4.

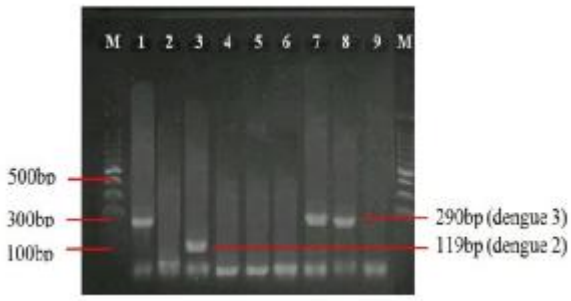

Figure 1.
The results of electrophoresis to detect dengue virus serotypes of endemic areas (samples 1-3), sporadic (samples 4-6) and potential (samples 6-9). As well as M: marker 100bp DNA *ladder*.

Serotype distribution of dengue virus in endemic, sporadic and potential areas of dengue in Kulon Progo Regency can be seen in Table 3.

Table 3 shows the serotypes of dengue virus 2 and 3 were found in endemic areas (Dipan, Wonosidi Lor), dengue virus 3 was found in potential areas (Panjatan and Kanoman), and while in sporadic areas there was no dengue virus serotype.

Table 3
Distribution of dengue virus serotypes in adult Ae.aegypti

Location		Serotypes of Dengue Virus
Endemic area		
Sub-district Wates	Wonosidi Lor	Dengue 3
	Gadingan	-
	Dipan	Dengue 2
Sporadic area		
Sub-district Wates	Driyan	-
	Kriyanan	-
	Durungan	-
Potential area		
Sub-district Panjatan	Panjatan	Dengue3
	Kanoman	Dengue 3
Sub-district Temon	Janten	-

Ae. aegypti larvae resistance test against Organophosphate (temefos)

The study found the larvae of *Ae. aegypti is* still vulnerable / sensitive to temefos in the endemic, sporadic and potential areas. Only 3 hamlets have decreased their vulnerability to tolerant status, namely Durungan, Kriyanan and Panjatan and there are no areas with resistant status. This result may be greatly influenced by operational factors which result in the low number of temefos usage. People consider temefos to be poisonous, so it is not applied to water reservoirs for consumption. In addition, some residents raise fish in water storage tanks to control mosquito larvae (Martini et al., 2019),(Singh et al., 2011).

Different studies conducted in Brazil by Lima et al.12 and Surabaya by Mulyatno et al. (Mulyatno et al., 2012) show that resistance to temefos insecticide has occurred in several sub-districts in the city of Surabaya with a mortality rate below 80%. This difference is based on differences in the use of temephos in each region. According to Pimsamarn et al, (Pimsamarn et al., n.d.) temefos 1% is recommended for larval control because the oral toxicity for mammals is very low (8600mg / kg male rats). In addition, it is quite good to be applied in water reservoirs because it has slow release act with low solubility in water (0.03mg / liter at 250C). These characteristics support natural selection from tolerant to high resistance when the material is applied under a mortality dose.

Continuous application without replacement with other insecticides for a long time will accelerate the development of resistant populations. According to Melo-Santos *et al.* (Melo-Santos et al., 2010) levels of temefos resistance in *Ae.aegypti* mosquito population in the outside environment is higher than the colonized in the laboratory. In addition, the residual effect of temephos larvicide, which is usually up to 3 months, becomes shorter (± 3 weeks) with an interval of 8 weeks. The resistance will be even higher if insecticides have been applied for 13 years (Montella et al., 2007),(Lima et al., 2011) In addition, the ease of obtaining temefos (abate) allows the use of this insecticide to be poorly monitored, whereas regular monitoring is important to determine the occurrence of resistance.

Adult Ae. aegypti Resistance Test against Synthetic Pyrethroids (cypermethrin)

All areas have a resistant status with the mortality percentage of adult mosquitoes below 80% (Table 4), so based on the criteria of Davidson and Zahar in 1973 the results of this study indicate that Ae aegypti mosquitoes from endemic, sporadic and potential areas in Kulon Progo Regency are already resistant to sipermethrin. The same thing was found in a study conducted in Thailand (Sirisopa et al., 2014) and a study conducted by Ahmad et al (Ahmad et al., 2007) which stated that the Ae aegypti mosquito in several regions in Indonesia was resistant even though the level of resistance was still low. However, Mulyamimgsih et al. found that Ae aegypti mosquitoes in several areas in Central Java were still susceptible to sipermethrin (Mulyaningsih et al., 2019).

Resistance caused by chemical insecticides occurs due to 2 main mechanisms, namely due to structural changes at the *target-site* and increased detoxification activity of enzymes such as esterase, oxidase and *glutathione S-transferase*. The resistance mechanism by sipermethrin occurs by influencing the activation and inactivation of action potentials on the nerve cell membrane (Nav Channel) which causes the continuation release of sodium ions on motor neurons and axons resulting in paralysis (knock down) and death in mosquitoes (Lima et al., 2011),(Paine & Brooke, 2016) Resistant mosquito populations were also found in sporadic and potential (free) areas. This is probably due to cross resistance between synthetic pyrethroids and organophosphates. The resistance to these two insecticides is due to the activity of detoxifying enzymes (oxidase, esterase A and esterase B) in accordance with the results of several studies which state that increased detoxification enzyme activity is associated with the increasing of resistance status against organophosphate and pyrethroid insecticides (Ahmad et al., 2007),(Paine & Brooke, 2016). In addition, the sampling location close to the rice fields allows for cross-resistance with agricultural pesticides (Paine & Brooke, 2016).

Determination of Dengue Virus Serotype

The RT-PCR method provides a rapid and sensitive diagnosis of dengue virus serotype. The RT-PCR method in insects is preferable to avoiding the false negative results

that are usually found in enzymatic methods (Urdaneta et al., 2005),(Lanciotti et al., 1992). In Indonesia, DEN-3 is associated with severe dengue cases and is the most widely distributed serotype, followed by DEN-2, DEN-1, and DEN 4 (Harapan et al., 2019). According to Urdaneta et al. (Urdaneta et al., 2005) DEN-3 is also a serotype with a high incidence in endemic areas in Venezuela and rapidly spreading, especially in areas without a previous incident of dengue virus even in areas that have never detected the virus (for 34 years).

In this study, 3 sporadic areas sampled had no detectable virus serotypes. This is probably due to the small amount of virus contained in the mosquito's thorax so that at PCR the DNA bands did not appear. In line with research conducted by Yasmon et al (Yasmon et al., 2010) that the false negative results when PCR occurred due to the very low number of virus particles in the mosquito samples. As stated by Urdaneta, et al (Urdaneta et al., 2005), a large number of samples to detect virus serotypes is important to avoid failure of viral DNA amplification due to the small number of viruses in the mosquito tissue.

In potential areas, DEN-3 virus serotypes were found, although there were never any DHF cases reported. This situation may occur due to imports from other areas which occur due to the very high mobility from endemic areas to potential areas In addition, viremia patients are rarely reported as cases because they do not show symptoms or only mild fever symptoms. In fact, viremia sufferers are the most dangerous transmitters because they are free to do activities in other areas including potential areas (Urdaneta et al., 2005). In addition, people with viremia are rarely reported as cases because they are asymptomatic or only with mild fever. In fact, viremia sufferers are the most dangerous transmitters because they are free to do activities in other areas including potential areas (Urdaneta et al., 2005)(Urdaneta et al., 2005). Virus serotypes will be detected earlier in mosquitoes than in humans as long as the number of mosquito pooling is large. Research in Venezuela found dengue virus serotypes in mosquitoes could be detected 8 weeks before the disease spreads, while in Singapore it was detected 6 weeks before the outbreak (Urdaneta et al., 2005).

Conclusions and Recommendations

The resistance status of Aedes aegypti in endemic, sporadic and potential areas to organophosphate insecticides (temefos) is in the susceptible to tolerant range, while the all areas status to synthetic pyrethroids (cypermethrin) are resistant. The dengue virus serotypes in endemic areas were dengue 2 and dengue 3. The potential areas were dengue 3, while in sporadic areas there were no virus serotypes.

Declaration of Conflicting Interests

The authors declared that no potential conflicts of interests with respect to the authorship and publication of this article.

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