RESISTANCE DETECTION OF Aedes aegypti *LARVAE TO CYPERMETHRIN FROM ENDEMIC AREA IN CIMAHI CITY WEST JAVA*

Deteksi Resistensi Larva *Aedes aegypti* terhadap *Cypermethrin* dari Daerah Endemis di Kota Cimahi Jawa Barat

Endang Puji Astuti[1*], Mara Ipa[1], Firda Yanuar Pradani[1]

[1]Loka Litbang P2B2 Ciamis, Badan Litbang Kesehatan, Kemenkes RI
Jln. Raya Pangandaran Km.03 Ds. Babakan Pangandaran, West Java, Indonesia.

Abstract*. Vector control programs using chemical insecticide e.g organochlorin, organophosphate, carbamate, and pyrethroid (cypermethrin). When those insecticides were applied continuously, it may lead to vector resistance. The aim of this research was to detect any resistance of* Ae. aegypti *to cypermethrin in endemic areas of Cimahi. This research is a laboratory study that used biochemical test which referred to Lee's method. Larva samples were collected from 8 villages, which are endemic area. Samples of larvae were collected from 15 villages belonged to dengue endemic areas in town of Cimahi, however, villages that meet the availability of larvae were only 8 villages. To detect the activity of monooxygenase enzyme, a biochemical assay was used in this research by created a reaction between larvae homogenate and sodium acetate substrate. The results of reaction were read using ELISA reader with spectrophotometer wave length of 595 nm. Overall, the results showed that most of the larvae in eight villages of Cimahi is still susceptible to cypermethrin. However, larvae from Cibabat village were 4% resistant, 2% tolerant, and 94% susceptible. On the other hand, Cigugur village showed that 12.7% larvae were tolerant and 87.3% still susceptible. Other villages like Cimahi, Cibeureum, Melong, Baros, Cipageran, and Pasirkaliki still remains susceptible. Resistance detection using biochemical assay of cypermethrin insecticide for* Ae.aegypti *resulting data stated that in 6 villages were still susceptible but in 3 other villages were already tolerant and 1 village was already resistance.*

Keywords: Aedes aegypti*, resistance, biochemical assay, cypermethrin, Cimahi, West Java*

Abstrak. Program pengendalian vektor dengan menggunakan insektisida kimiawi yaitu organoklorin, organofosfat, karbamat, dan piretroid secara terus-menerus dapat menyebabkan resistensi. Tujuan dari penelitian ini adalah untuk mendeteksi status resistensi larva *Ae*. *aegypti* terhadap insektisida *cypermethrin* di daerah endemis Demam Berdarah *Dengue* (DBD) Kota Cimahi. Jenis penelitian ini adalah penelitian skala laboratorium dengan uji biokimia menggunakan metode Lee. Sampel larva dikoleksi dari 15 kelurahan yang termasuk daerah endemik DBD di Kota Cimahi, namun yang memenuhi ketersediaan larva hanya delapan kelurahan.Untuk mendeteksi aktivitas enzim monooxigenase dilakukan pengujian secara biokimiawi dengan mereaksikan homogenat larva *Ae*. *aegypti* dengan substrat sodium acetat. Hasil reaksi diperoleh menggunakan ELISA *reader* dengan *spectrophotometer* dengan panjang gelombang 595 nm. Secara keseluruhan hasil penelitian menunjukkan sebagian besar larva di delapan kelurahan kota Cimahi masih bersifat rentan terhadap insektisida *cypermethrin*. Namun, larva yang berasal dari kelurahan Cibabat 4% sudah resisten, 2% sudah toleran, dan 94% rentan. Berbeda dengan di Kelurahan Cigugur yaitu 12,7% larva menunjukkan gejala toleran dan 87,3% rentan. Enam kelurahan lainnya yaitu Cimahi, Cibeureum, Melong, Baros, Cipageran, dan Pasirkaliki masih rentan. Deteksi resistensi dengan uji biokimia pada larva *Ae*. *aegypti* terhadap insektisida *cypermethrin* di enam kelurahan masih rentan, sedangkan 3 kelurahan lainnya sudah menunjukkan gejala toleran dan satu kelurahan menunjukkan sudah resisten.

Kata Kunci*: Aedes aegypti,* resistensi*, cypermethrin,* Cimahi, Jawa Barat

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* Correspondence: puji_yn@yahoo.co.id; Tel./Faks.: (+62 265) 639375

BACKGROUND

Dengue is "*vector-borne viral*" viruses that remain to be a serious transmitted disease. *Aedes aegypti* is implicated as primary vector for dengue, which exist mainly in tropical areas[1], *Aedes albopictus* acted as a secondary vector. In Indonesia, the number of Dengue Haemorrhagic Fever (DHF) cases each year tends to increase and spreads widely. At the beginning of 2004, several outbreaks of DHF occurred in several provinces in Indonesia such as: Central Java, East Java, Bali, Jakarta, West Java, East Nusa Tenggara (NTT), West Nusa Tenggara (NTB), and South Kalimantan.[2]

Until 2007, all the districts in West Java Province have reported the incidence of dengue outbreaks. Results of secondary data cases analysis were 1080 villages have the status of endemic dengue.[3] Cimahi is an urban area with relatively dense population and high mobility. Entire village in the city of Cimahi have reported cases of DHF, this is due to ease of transportation, increasing population and lack of awareness of public health it self.[4]

Fogging with insecticides is usually used in outbreaks, especially for cases of DHF. The purpose of this activity is to control *Ae. aegypti* adults by interrupt transmission mechanism. Until now fogging is still the main choice for dengue vector control during outbreaks. This effort will be effective if the targeted mosquito is not resistance to insecticides that has been used.

Vector control programs at this time are generally still using four groups chemical insecticides such as organochlorine, organophosphates, carbamates, and pyrethroids. The use of pyrethroid insecticides in recent years have shown a proliferation, several types of organochlorine and organophosphat compounds which are more toxic have decreased.[5] Some cases of resistance were also reported in Indonesia, one mosquito vector of malaria in Central Java and Yogyakarta also susceptible to organophosphate and carbamate insecticides.[6]

Pyrethroid clasified as moderate toxic. Some insecticides from this group e.g: *d-allethrin*, *transflutrin*, *bioallethrin*, *pralethrin*, *d-phenothrin*, *cyphenothrin* atau *esbiothrin* dan *cypermethrin*. Cypermethrin was contact poison and stomach poison that have been used generally from farming, husbandry and agriculture industry. Cypermethrin becoming popular because its cheap and effective.[4]

In Cimahi, since the year of 2002, the use of insecticides has shifted from the class of organophosphates to pyrethroids in population vector control for areas with endemic dengue. The area which pyrethroid insecticides were used in a period of more than 3 years required test vector susceptibility to DHF.[7] The purpose of this study was to detect resistance of *Ae. aegypti* to cypermethrin in endemic areas in Cimahi city.

The aim of research is to identify in which areas has resistant vector to cypermethrin in Cimahi and this result can be used by stake holder to make a decision about which insecticides can still be use as vector control.

MATERIAL AND METHOD

This research is a laboratory study, dengue endemic villages mosquitos' samples were taken in the town of Cimahi. Biochemical tests performed at the Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Central Java.

This research was conducted in four months from August to November 2010 while the chance of rainfall was high. The sample unit was individual members of a population from 15 villages. However, because of the availability of insufficient number of larvae in seven villages, only eight village larvae were tested biochemically. Larval samples

collected from 20 houses selected in each village, which focused on household breeding place of *Ae. aegypti* larvae. Each village took fourth instar of 47 larvae, so the total number of larvae used for biochemical tests were 376 larvae.

The larvae found were taken by using a pipette, then inserted into the container for larvae. Next, larvae collected were taken to insectarium of Vector Borne Disease Control Research and Development Office in Ciamis for rearing until third fertilization (F_3). Larvae used were fourth instar larvae from the third fertilization (F_3). Biochemical test was conducted on the enzyme activity monooxigenase.

Monooxigenase enzyme activity assay based on the method of Lee.[8] Fourth instar larvae individually created homogenate filtrate in 20 mL and 80 mL potassium phosphate buffer. This work was performed with two replicates of each sample of larvae. Substrate solution was prepared immediately by mixing 0,01 g TMBZ + 5 mL of methanol + 15 mL sodium acetat buffer. The prepared substrate solution is added with approximately 200 mL into the wells of micro plate, followed by the addition of 3% H_2O_2 for 25 mL. Microplate and its contents incubated at room temperature for 2 hours.

The emergences of white color indicating the mosquitoes are still susceptible while the blue color indicating the mosquitoes resistant to cypermethrin. Results subsequently read by spectrophotometer at a wave length of 595 nm. Interpretation of the results of ELISA reader with spectrophotometric value shown of level monooxygenase which expressed as optical density (od). This reader is categorized into three categories which is (1) susceptible (SS) if od < 2:00 are shown in white color; (2) tolerant (RS) if value 2.0-2.5 are shown in light blue color; (3) resistant (RR) if value 2.6-3.0 (resistant/RR) shown with dark blue color.[8] Data was analyzed descriptively.

RESULTS

The collection of *Ae. aegypti* larvae was conducted in 15 villages in Cimahi, but only eight villages were sufficient in larvae availability for biochemical testing. These villages are Cibabat, Cibeureum, Cipageran, Cigugur, Baros, Melong, Pasirkaliki, and Cimahi. Larvae in other villages did not succeed breeding because there were not enough larvae, and all larvae eventually died.

Monooxygenase enzyme test results (Cytochrom P_{450} assay) with a wavelength of 595 nm indicates that most individuals of *Ae. aegypti* in the city of Cimahi remains susceptible (SS) of cypermethrin which is concluded from 97.34% of the total larvae that has been tested (376 larvae). There are three locations that showed a decrease in susceptibility in Cibabat, Cigugur, and Baros villages, individual larvae in these locations are tolerant (RS). Different result was shown in Cibabat village, where only one case obtained that was resistant (RR) larvae which is 0.27% of the total larvae tested.

Table 1. Level of Resistance *Aedes aegypti* Larvae

Location	Susceptible (n = 366)		Tolerant (n = 9)		Resistance (n = 1)	
	n	%	n	%	n	%
Cibabat	44	94	2	4	1	2
Cigugur	41	87.3	6	12.7	0	0
Melong	47	100	0	0	0	0
Pasirkaliki	47	100	0	0	0	0
Cipageran	47	100	0	0	0	0
Cimahi	47	100	0	0	0	0
Baros	46	98	1	2	0	0
Cibeureum	47	100	0	0	0	0

Remark : Percentage for each location

DISCUSSION

Fogging with insecticides used only in emergency situation such as outbreak of dengue fever. Fogging purpose was to killed

adult *Ae. Aegypti* to cut of transmission these disease. Until today, fogging still become most popular choice to control outbreak DHF. These efforts will be effective if the target mosquitoes have not resistant yet to insecticides been used.

Results showed that most of *Ae. aegypti* in Cimahi, are still susceptible. There are three villages, Cibabat, Cigugur, and Baros which showed that larvae of *Ae. aegypti* has been tolerant and only one cases in Cibabat was resistant.

Only one case of resistance and some case of tolerance that has been found in Cimahi, presumably because fogging was done continuously in those areas. This happens because the mosquito of *Ae. aegypti* and other dengue vector systems are able to develop immunity to insecticides that are often used.[9] Since the year 2002, cypermethrin (*pyrethroid*) insecticide has been used for fogging in this area.[4]

The similar study conducted by Wahyudin, 2009 reported that *Ae. aegypti* originating from the city of Cimahi were resistant to organophosphate and tolerant to pyrethroid.[7] Research conducted by Mardihusodo, 1996 reported that *Ae. aegypti* in Yogyakarta still susceptible based on biochemical enzymatic test, but the mosquito has the potential to become resistant to insecticides associated with the activities of non-specific esterase enzyme that is able to hydrolyze α-naphtylacetate.[10] In 2009, level of resistance of *Ae. aegypti* in the city of Yogyakarta, is still in susceptible status (RR) of 96.7% and 3.3% showed tolerant (RS) to pyrethroid with a mechanism of increased enzyme activity monooxigenase.[11]

This study used larvae of F_3 for biochemical test which means population of the less dominant resistance gene was expected to be detected than first fertilization (F_1). Inheritance of this resistance gene is similar to the study of insects *P. xylostella* against pyrethroid, in which male mating resistant and susceptible females will produce fertilization that have 100% susceptible heterozygot genotype zygote heterozygote. First fertilization (F_1 RS) when mating with susceptible female has fertilization zygote genotype of 50% heterozygote susceptible and 50% homozygote susceptible, with 100% susceptible phenotypes.[12]

There are two mechanism of action for pyrethroid insecticides important things which are an increase in detoxification and insensitivity towards the target organ. Although, the changes of esterase, glutathione transferase, and monooxygenase has a different effect, the knock down resistance (Kdr) of the insects have been detected.[13] According to Hemingway, several gene mutations insect voltage gated sodium channels have been identified and shown to Kdr that is associated with pyrethroid insecticides.

Pyrethroid is still widely used as insecticides for vectrol control, because of the high toxicity on the vector target and relatively low adverse effect on mammals and human. However, after routine application, many field vector populations have been developed tolerant and resistant to this insecticide.[14] Thus, insecticide dose selection and rotation of insecticide classes is needed to obtain the optimal result in accordance with resistance management.[15]

CONCLUSION

Most of the *Ae. aegypti* larvae tested in Cimahi city were still susceptible to cypermethrin (pyrethroid), only one individual larvae showed that have a resistant status which found in Cibabat village. Larvae that showed tolerance symptoms were found at Cibabat, Cigugur, and Baros villages, Cigugur have higher number of tolerant larvae than other villages.

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