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Current Resistance Status to Synthetic Pyrethroid Insecticides in *Aedes aegypti* (Diptera: Culicidae) Populations from Different regions of Bogor City, Indonesia

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Abstract
Dengue fever (DF) Aedes aegypti (L.) is a primary vector of dengue fever in Bogor City in Indonesia. The use of pyrethroid insecticides are commonly used in the vector control program. The current research evaluatedthe distribution of the DF cases in relation tothe insecticide resistance status of the vector to deltamethrin, apyrethroid compound. Ae. aegypti populations were collected in 35 villages by using ovitraps, the collected eggs were reared in the laboratory for2 generations, and susceptibility tests to deltamethrin were conducted on unfed female mosquitoes 3 to 5 days old. WHO bioassays were performed with impregnated papers with 0.025% deltamethrin. Deltamethrin resistance was detected in 30 (85.7%) Ae. aegypti populations (Baranang Siang, Katu Lampa, Lawang Gintung, Tegal Lega, Kebon Kelapa, Cilendek Barat, Menteng, Kebon Pedes, Kedung Waringin, Bantar Jati, Tegal Gundil, Kayu Manis, Ciluar, Kedung Halang, Ciwaringin, Panaragan, Pamoyanan, Semplak, Sindang Rasa, Sindang Sari, Cikaret, Cipaku, Empang, Pasir Mulya, Loji, Sempur, Tanah Baru, Cimahpar, and Kencana strains); 4 (11.4%) populations (Sukadamai, Harjasari, Kertamaya and Situ Gede) were tolerant, and one (2.9%) population was susceptible (Cibadak). The current study clearly shows that the resistant strain against deltametrin was found in the most Ae. aegypti strain in Bogor City. This informations would help in formulating the strategy of vector control, especially in management of insecticide resistance and insecticide policy as well.
Keywords: *Aedes aegypti, pyrethroid resistance, deltamethrin, Indonesia*.

Introduction

Dengue Hemorrhagic Fever (DF) is a health problem that is a major concern in Indonesia. From 1968 until 2009, DF cases in Indonesia were belong to the first ranks with the highest number of people affected in Southeast Asia (Gubler *et. al.*, 1978).[1]

The dengue viruses are transmitted by rough *Aedes aegypti* (L.) and *Ae albopictus* (Skuse). Vector control programs were conducted by Ministry of Health and also by Non Goverment officials, however, the number of patients with DF has increased over time. Bogor City is one of DF endemic area in West Java, Indonesia. Severeal vilages such as Bantar Jati, Kedung Halang, Kayu Manis, Tegal Gundil, and Sukadamai, were designated as high endemic areas of DF every year (Bogor City Health Office, 2012).[2] The high rate of DF cases in Bogor likely caused by high mobility of inhabitans, high density populations, poor infrastuctures in some areas, unhygiene behavior, unwise insecticide applications for vector control, and perhaps climate change.

Adulticide applications are the most common method for DF vector control. However, the applications of insecticides sooner or later will cause tolerance and resistance in the target organism. Reports on tolerance and resistance of *Ae. aegypti* to the insecticide malathion, bendiokarb and deltamethrin havebeen widely reported. In Malaysia, *Ae. aegypti* was reported as highly resistant to malathion with the resistance ratio reaching 52.7 (Hidayati *et al.*, 2011).[3] In Indonesia, Widiarti et al. (2011) reported that *Ae. aegypti*was resistant to malathion 0.8%, bendiocarb 0.1%, lamdacyhalothrin 0.05%, permethrin 0.7%, deltamethrin 0.05% and etofenfrox 0.5% in Central Java and Yogyakarta, but some areas were still susceptible to cypermethrin 0.05%, and bendiocarb 0.1%.[4] Shinta *et al.*(2008) also reported that *Ae. aegypti* was resistant to malathion 0.8% and lamdacyhalothrin 0.05%, but still tolerant to lamdacyhalothrin 0.25% and susceptible to malathion 5% in Jakarta and Bogor (Tanah Sareal).[5] Nusa *et. al.* (2008) reported that *Ae. aegypti*was still susceptible to malathion 0.8% in Depok.[6] Boewono *et. al.* (2012) reported that *Ae. aegypti*, dengue vector in Bontang City, East Kalimantan were found to be resistant to malathion and lambdacyhalothrin.[7] The presence of tolerance and resistance of *Ae. aegypti* populations to adulticides may have contributed to the increase in the cases of dengue fever in the city of Bogor. Insecticide aplications in Bogor through the program of fogging-focus in the DF cases area to eliminate adult infective vectors were conducted prior to 2000 using malathion.After 2000 the malathion was replaced by pyrethroid (Bogor City Health Office 2012).[2] However, no comprehensive research was done to determined the distribution of DHF cases and also the insecticide resistance status of *Ae. aegypti* in Bogor. The objective of the present study was to evaluate the insecticide resistance status of the DF vector to deltamethrin. The results of this study are expected to provideinformation useful in controlling *Ae. aegypti* in Bogor City.

Material and Methods

Mosquito Sampling

This research was conducted in two stages, the sampling of eggs of *Ae. aegypti* from the field, and the testing of susceptibility status. *Ae. Aegypti* eggs were collected from 35 villages in 6 subdistricts of Bogor city during June - July 2014, and June - July 2015. The geo-coordinates of collection sites in Bogor City were presented in Table 1. Five houses in each village was selected randomly for placing the ovitraps for 5-6 days to collect the mosquito eggs. The eggs were reared in the Insectarium of Faculty of Veterinary Medicine, Bogor Agricultural University. F2 progeny were used for testing.

Susceptibility Testing

Susceptibility testing used the standard method of WHO (2009).[8] The insecticides used for susceptibility test was deltamethrin 0.025 % in the form of insecticide impregnated paper.The susceptibility test was done under ambient room temperatures of 27-28°C and relative humidity of 75-90%. The number of mosquitoes used were 20 *Ae. aegypti* from each location. The susceptible strain of mosquito used for control came from the Laboratory of Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University. The mosquitoes were put into holding tubes , then transferred into exposure tubes coated by deltamethrin insecticide-treated papers for 60 minutes. After that, all the mosquitoes were transferred back to the holding tube. The mortality rate was observed at 1, 2, 3, 4, 5, 6, 12 and 24 hours after exposures. The dead mosquitoes were unable . If the mosquito mortality in the control group was greater than 20% then it should be re-tested and if the morality occurred between 5-20% then the data must be corrected by Abbot formula (WHO, 1975).[9]

Data Analysis.

Susceptibility or resistance status was determined based on the percentage of mosquito mortality. When mortality was below 80%, the population was declared resistant, between 80-97% was tolerant, and between 98-100% was susceptible (Herath, 1997).[10]The result then was analyzed using probit analysis to determine LT_{90} (lethal time 90). The resistance level (RR_{90}) was determined by comparing the LT_{90} values of mosquito tested to the LT_{90} values of Laboratory strain (Ahmad *et. al*., 2007).[11]

Results

The presence of tolerance and resistance of *Ae. aegypti* population of Bogor City to deltamethrin was presented in Table 2 and Fig 1. Based on percentage mortality, high mortality 24 hour post contact was found in Cibadak strain (98.35%), and followed Situ Gede (97.33%), Kertamaya (96%), Sukadamai (92%), and Harjasari (85.33%) strains, the lowest mortality were occurred in Bondongan (0%), Menteng (0%), Kebon Pedes (0%) and Kedung Waringin (0%) strains.For comparison, the mortality rate of the susceptible laboratory strain was 100% to deltamethrin (Table 2).

Based on criteria of Herath (1997), 30 (85.7%) populations of*Ae aegypti* (Baranang Siang, Katu Lampa, Lawang Gintung, Tegal Lega, Kebon Kelapa, Cilendek Barat, Menteng, Kebon Pedes, Kedung Waringin, Bantar Jati, Tegal Gundil, Kayu Manis, Ciluar, Kedung Halang, Ciwaringin, Panaragan, Pamoyanan, Semplak, Sindang Rasa, Sindang Sari, Cikaret, Cipaku, Empang, Pasir Mulya, Loji, Sempur, Tanah Baru, Cimahpar, and Kencana strains) were resistant to deltamethrin, with mortality <80%. In addition, the other 4 (11.4%) strains (Sukadamai, Harja Sari Situ Gede and Kertamaya) showed tolerance status, with mortality rate between 80 - 97% (Table 2). Most populations tested had developed tolerance and resistance to deltamethrin. Only one susceptible (2.9%) strain (Cibadak strain) was found from Bogor City. Distribution of the resistant status of *Ae. aegypti* against deltamethrin in Bogor City was shown in Figure 1.

The LT_{90} values of *Ae. aegypti* were reached in 1.65 hour for the susceptible laboratory strain, and in 3.,89 hours for Sukadamai strain, 4,50 hour for Situ Gede strain, and 6.85 hour for Kertamaya strain. The LT_{90} were varied in all resistant strains, such as strains of Sindang Sari (38,70 hours), Ciwaringin (54,67hour), Kedung Halang (70,45 hour), Sindang Rasa (82,28 hour), Cilendek Barat (181.39 hour), Tega Lega (220.82 hour) and Baranang Siang (327.64 hour).

The resistance level (RR_{90}) for each resistant strain of *Ae. aegypti* in several area of Bogor City to deltamethrin were also varied (Table 2). The result showed that Bondongan, Menteng, Kebon Pedes, Kedung Waringin strains were the most resistant strain with RR_{90} unlimited, then followed by Cikaret (RR_{90} = 83.20), Empang (RR_{90} = 57.14), Semplak (RR_{90} = 55.61), Sindang Rasa (RR_{90} = 49,99), Loji (RR_{90} = 47,92), Kedung Halang (RR_{90} = 42,80), Kencana (RR_{90} = 40.59), Ciwaringin (RR_{90} = 33,21), Kayu Manis (RR_{90} = 30,40), Pamoyanan (RR_{90} = 27,43), Sindang Sari (RR_{90} = 23,51), Harjasari (RR_{90} = 11,24), Ciluar (RR_{90} = 3,33), Situ Gede (RR_{90} = 2,74) and Suka Damai (RR_{90} = 2,36). In this result, based on the RR_{90}, it can be determined that 15 strains of *Ae. aegypti* was tolerance with RR_{90} less than 10, and 20 strains were resistance againts deltamethrin with RR_{90} more than 10.

Table 1 Location and geo-coordinates of *Aedes aegypti* collection sites in Bogor City

Subdistrict	Village	GPS Coordinates	Altitude (m)
Tanah Sareal	KebonPedes	S 06°33.942' , E 106°48.093'	121
	KedungWaringin	S 06°33.876' , E 106°46.728'	111
	KayuManis	S 06°32.087' , E 106°46.123'	182
	Sukadamai	S 06°33.158' , E 106°47.493'	148
	Kencana	S 06°39.011' , E 106°49.633'	137
	Cibadak	S 06°32.593' , E 106°46.180'	295
Bogor Utara	Bantarjati	S 06°34.486' , E 106°48.565'	309
	TegalGundil	S 06°34.473' , E 106°48.788'	228
	Ciluar	S 06°33.550' , E 106°49.591'	195
	KedungHalang	S 06°33.659' , E 106°48.746'	175
	Tanah Baru	S 06°35.119' , E 106°49.155'	222
	Cimahpar	S 06°35.248' , E 106°49.709'	138
Bogor Tengah	KebonKelapa	S 06°35.521' , E 106°47.125'	283
	TegalLega	S 06°35.904' , E 106°48.520'	215
	Ciwaringin	S 06°35.036' , E 106°47.392'	257
	Panaragan	S 06°35.810' , E 106°47.088'	315
	Sempur	S 06°35.365' , E 106°48.030'	306
Bogor Selatan	Bondongan	S 06°36.643' , E 106°48.093'	350
	LawangGintung	S 06°37.430' , E 106°48.778'	313
	Pamoyanan	S 06°37.678' , E 106°48.544'	419
	Harjasari	S 06°39.200' , E 106°50.699'	483
	Cikaret	S 06°37.125' , E 106°47.088'	293
	Cipaku	S 06°38.172' , E 106°48.815'	194
	Empang	S 06°36.557' , E 106°47.655'	273
	Kertamaya	S 06°39.014' , E 106°49.634'	349
Bogor Barat	Cilendek Barat	S 06°34.281' , E 106°46.085'	207
	Menteng	S 06°34.695' , E 106°46.634'	224
	Semplak	S 06°33.802' , E 106°45.809'	189
	Situ Gede	S 06°33.089' , E 106°44.420'	263
	PasirMulya	S 06°35.754' , E 106°46.638'	162
	Loji	S 06°35.015' , E 106°46.223'	114
Bogor Timur	Baranangsiang	S 06°36.201' , E 106°48.203'	350
	Katulampa	S 06°37.366' , E 106°49.432'	136
	Sindang Rasa	S 06°38.420' , E 106°50.354'	295
	Sindang Sari	S 06°39.187' , E 106°50.768'	439

Table 2 Resistance Status to Sintetic Pyrethroid Insecticide in *Aedes aegypti* Populations from 35 regions of Bogor City2014-2015

Number of Strain	Strain of *Ae. aegypti*	Deltamethrin 0.025%			
		Mortality (%)	Susceptibility Status	LT_{90} (hour)	RR_{90}
1	KebonPedes	0,00	Resistant	∞	∞
2	KedungWaringin	3,33	Resistant	∞	∞
3	KayuManis	76,00	Resistant	50,11	30,40
4	Sukadamai	92,00	Tolerant	3,89	2,36
5	Kencana	16,65	Resistant	92,26	40,59
6	Cibadak	98,35	Susceptible	∞	∞
7	Bantarjati	6,67	Resistant	226,38	0,78
8	TegalGundil	8,33	Resistant	107,68	0,37
9	Ciluar	70,67	Resistant	5,47	3,33
10	KedungHalang	56,00	Resistant	70,45	42,80
11	Tanah Baru	77,32	Resistant	21,41	9,42
12	Cimahpar	16,65	Resistant	89,34	39,30
13	KebonKelapa	1,67	Resistant	60,06	0,21
14	TegalLega	43,33	Resistant	220,82	0,76
15	Ciwaringin	66,67	Resistant	54,67	33,21
16	Panaragan	74,67	Resistant	4,80	2,92
17	Sempur	52,50	Resistant	36,34	15,99
18	Bondongan	0,00	Resistant	∞	∞
19	LawangGintung	8,33	Resistant	173,87	0,60
20	Pamoyanan	65,33	Resistant	45,14	27,43
21	Harjasari	85,33	Tolerant	18,50	11,24
22	Cikaret	10,00	Resistant	189,29	83,28
23	Cipaku	8,35	Resistant	78,46	34,52
24	Empang	10,00	Resistant	129,87	57,14
25	Kertamaya	96,00	Tolerant	6,85	3,01
26	Cilendek Barat	78,33	Resistant	181,39	0,62
27	Menteng	8,33	Resistant	∞	∞
28	Semplak	56,00	Resistant	91,54	55,61
29	Situ Gede	97,33	Tolerant	4,50	2,74
30	PasirMulya	28,35	Resistant	66,65	29,32
31	Loji	13,35	Resistant	108,93	47,92
32	Baranangsiang	20,00	Resistant	327,64	1,12
33	Katulampa	38,33	Resistant	236,26	0,81
34	Sindang Rasa	48,00	Resistant	82,28	49,99
35	Sindang Sari	80,00	Resistant	38,70	23,51
	Susceptible Laboratory Strain	100	Susceptible	1,65	

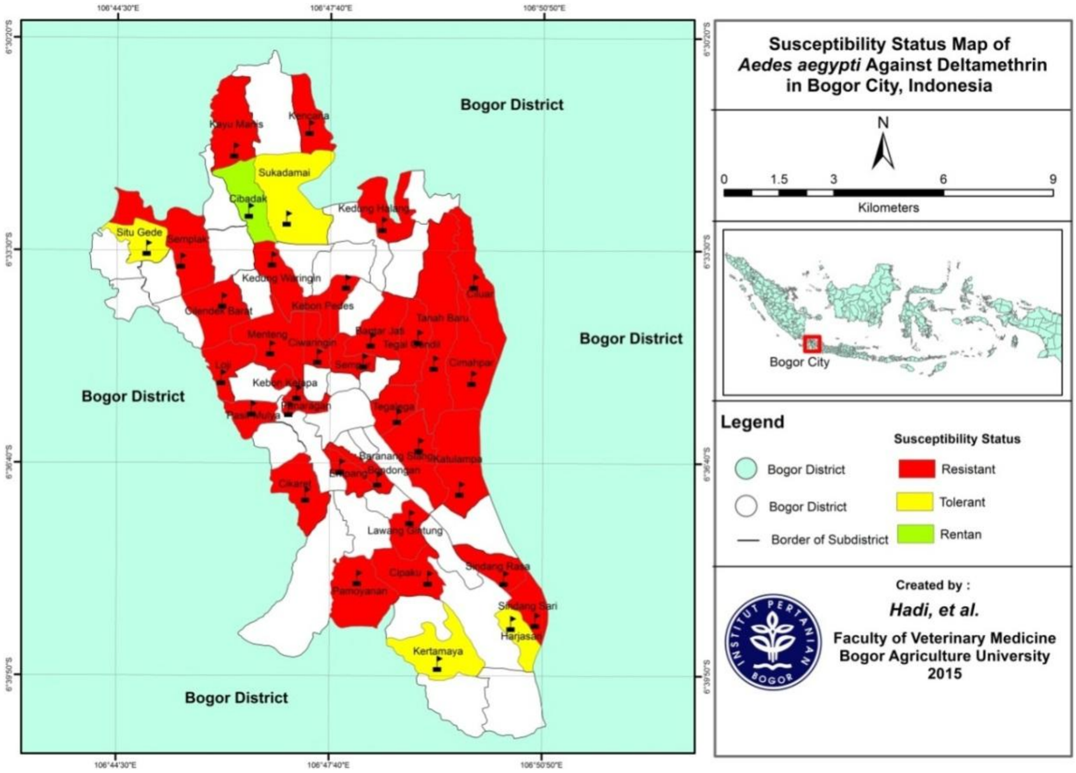

Figure 1. Map of Resistance Status to Sintetic Pyrethroid Insecticide in *Aedes aegypti* Populations from 35 regions of Bogor City2014-2015

Discussion

Based on mortality rates, 30 (85.7%) populations of *Ae. aegypti* in Bogor City were resistant, 4 (11.4%) strains tolerant, and one (2.9%) susceptible against deltamethrin 0.025%. These conditions might happen correlated with the past spray histories of insecticide used when the DF case increases. Many studies have shown that resistance level of a population can increase in several generations when selection using insecticides was applied to the population (Chareonviriyaphap *et. al.*, 2002).[12]In Bogor City, dengue cases appear each year fluctuates, the peak of cases occurred in 2010 and declined in 2014 (Bogor City Health Office 2014).[2]Actually, rainfall and humidity affected the incidence of dengue cases because of the availability of vector habitats due to rainfall occurs throughout the year. Similar condition of DF cases were reported in Semarang, Central Java (Mintarsih *et al.*,1996).[13] According Sugiarto *et. al.*(2016a) therainy season in Indonesia associated with the behavior of the people and the availability of habitat. In the rainy season (October to February), the people is more concentrated in the house activity (indoor), so they have high risk to contact with the mosquito vector which have indoor activity (*Ae. aegypti*). The irregular rainfall is also potentially increasing the availability of the vectorhabitats around the household. Sugiarto*et. al.* (2016b) stated the availability of water in the media will cause mosquito eggs hatch and after 10-12 days will turn into adult mosquitoes.[15] If human was biten by a mosquito infected with dengue virus then in 4-7 days then there will be symptoms of dengue. Based on the risk factor of rainfall in Indonesia, the starting point to enter the rainy season until the occurrence of DF incidence is approximately 3 weeks (MoH RI 2010).[16]

Pyrethroid insecticide is the most widely used by the public for residential vector and pest control.[17] The use of this insecticide in DF vector control programs (fogging) for human settlement in Bogor City have been done since 2000. Before 2000, malathion was used in fogging program (Bogor City Health Office 2012).[2] Currently the majority of household insecticides available in the markets of

Bogor also belong to pyrethroid class and one of them is deltamethrin. Deltamethrin is an insecticide that interferes with the nervous system by binding to proteins of voltage-gated sodium channels that regulate nerve impulses. Nerve impulses will undergo continuous stimulation and lead to insect tremors or excitation.

Valles *et.al*. (1997) stated that a population is considered resistant if the resistant ratio 90% (RR_{90}) is >10.[18] However, higher RR_{90}compared to laboratory strain suggested that those strains have already developed tolerance to deltamethrin and may develop resistance in the future. This is likely to happen if pyrethroids keep being used to control the population, thus allowing the resistant individuals to survive and reproduce. Sugiarto et. al. (2017) stated that the high diversity of species shown by the increasing number of mosquito species that dominate a population. High diversity of mosquito shows stable population, but in medical terms, it can be used as threat indicator.[19]

This observation needs further investigations to establish whether the vector species after the withdrawal of any other insecticide regains its susceptibility status. Further studies in other parts of the regions and determination of biochemical mechanisms involved in the development of insecticide resistance are in progress.

Conclusion

The result showed the current status resistance to deltamethrin in *Ae. aegypti* populations were shown in 30 (85.7%) strains (i.e. Baranang Siang, Katu Lampa, Lawang Gintung, Tegal Lega, Kebon Kelapa, Cilendek Barat, Menteng, Kebon Pedes, Kedung Waringin, Bantar Jati, Tegal Gundil, Kayu Manis, Ciluar, Kedung Halang, Ciwaringin, Panaragan, Pamoyanan, Semplak, Sindang Rasa, Sindang Sari, Cikaret, Cipaku, Empang, Pasir Mulya, Loji, Sempur, Tanah Baru, Cimahpar, and Kencana strains), and 4 (11.4%) strains (i.e. Sukadamai, Harjasari, Kertamaya and Situ Gede) were tolerant, and one susceptible (2.9%) strain was Cibadak strain. The current study clearly shows that the resistant strain against deltametrin was found in the most *Ae. aegypti* strain in Bogor City. This pyrethroid resistance was one of the cause of dengue cases in Bogor. This informations would help in formulating the strategy of vector control, especially in management of insecticide resistance and insecticide policy as well.

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