RESISTANCE OF *AEDES AEGYPTI* (L.) LARVAE TO TEMEPHOS IN SURABAYA, INDONESIA

Kris Cahyo Mulyatno[1,2], Atsushi Yamanaka[1,3], Ngadino[4] and Eiji Konishi[3,5,6*]

[1]Indonesia-Japan Collaborative Research Center for Emerging and Reemerging Infectious Diseases, [2]Laboratory of Entomology, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia; [3]Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Japan; [4]Environmental Health Academy, Surabaya, Indonesia; [5]Department of International Health, Kobe University Graduate School of Health Sciences, Kobe, Japan, [6*]BIKEN Endowed Department of Dengue Vaccine Development, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. The resistance of *Aedes aegypti* mosquitoes to insecticides threatens dengue virus control efforts. In this study, *Ae. aegypti* larvae collected from 12 subdistricts in Surabaya, Indonesia, where dengue is endemic, were tested for resistance to the organophosphate, temephos. Susceptibility testing, performed according to World Health Organization (WHO) methods, showed all field strains were resistant to temephos at a dose of 0.012 mg/l, with mortality rates at 24 hours of 22% to 60%. Another susceptibility test to determine median lethal time (LT_{50}) indicates resistance ratios ranging from 2.2 to 8.5. Although incipient resistance was detected at a dosage of 1 mg/l, as determined by the LT_{50}, mortalities higher than 80% within 24 hours were detected using the WHO method in nine subdistricts of Surabaya, indicating temephos at 1 mg/l is still effective in field conditions in these areas. In three subdistricts (Tambaksari, Gubeng and Sawahan), the mortality rates were under 80%, indicating possible resistance to temephos.

Keywords: *Aedes aegypti*, temephos, susceptibility test, Indonesia

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are two presentations of a mosquito-borne disease that causes public health problems in many tropical and subtropical areas. In Indonesia, the first reported dengue outbreak occurred in Surabaya and Jakarta in 1968 (Suroso, 1997). Surabaya, consisting of 31 subdistricts, is one of the largest cities in Indonesia, with approximately 2,000-3,000

Correspondence: Dr Atsushi Yamanaka, Institute of Tropical Disease, Airlangga University, Kampus C UNAIR, Jl Mulyorejo, Surabaya 60115, Indonesia.
Tel/Fax: +62-31-594-0917
E-mail: paradios99@yahoo.co.jp

*The "BIKEN Endowed Department of Dengue Vaccine Development" was endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

dengue cases annually, and 10 deaths being reported in the past 10 years (Surabaya Health Office, unpublished data). The main vector for dengue disease is *Aedes aegypti* (Linnaeus). Its close relative, *Aedes albopictus* Skuse, is also involved in dengue transmission as a secondary vector (Ministry of Health Indonesia, 1999).

Since an effective vaccine is not yet available, the prevention and control of dengue disease relies on vector control, mainly using insecticides (Suroso, 1997). The organophosphate temephos has been the main insecticide for mass larviciding (Ministry of Health Indonesia, 1999). Temephos is applied as 1% sand granules (Abate®) into household water containers and other breeding foci for larval control. However, long-term use of this chemical has been a factor in the development of resistance by *Ae. aegypti* (Ministry of Health Indonesia, 1999). Information regarding the susceptibility of *Ae. aegypti* to insecticides in Indonesia is limited.

MATERIALS AND METHODS

Mosquito collection

In the present study, *Ae. aegypti* larvae were collected from 12 subdistricts in Surabaya during 2009 and 2010. These larvae were collected from indoor water containers called Bak Mandi (containing approximately 1,000 liters) from 10 houses in each subdistrict.

Colonization

The collected larvae were grown in the laboratory and the emerged adults were used to identify the species. The larvae were placed in dechlorinated water in a pan 30 cm by 20 cm kept at a temperature of 28 ± 1ºC and relative humidity of 85 ± 5%. The emerged adult mosquitoes were maintained in a cage 30 cm x 30 cm x 30 cm and their eggs were used for producing the next generation. Larvae of the F1 and F2 generations were used for susceptibility testing.

Larval susceptibility testing

Larval susceptibility testing was performed according to the World Health Organization (WHO) method (WHO, 1981) with slight modification, using commercial Abate 1G® (Baden Aniline and Soda Manufacturing, Ludwigshafen, Germany) containing 1% temephos. The larvae were tested using temephos at a dose of 0.012 mg/l [diagnostic dose recommended by the WHO (1992); corresponding to 1.2 mg/l of Abate®) and 1 mg/l (operational dose used in Indonesia; corresponding to 0.1g/l of Abate®]. Four cups were run in parallel for each test. Each cup contained 30 late 3rd or early 4th instar larvae. Larval mortality was scored at the end of a 24-hour holding period. In another susceptibility test, larval mortality was recorded every 15 minutes for 2 hours and 24 hours after exposure, using a temephos dosage of 1 mg/l. Quadruplicate testing was carried out. The median lethal time (LT_{50}), defined as the time required to kill 50% of the larvae, was calculated using the Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS, College Station, TX). The resistance ratio was calculated by dividing the LT_{50} value obtained with the field strain by that obtained with the laboratory strain. The laboratory strain of *Ae. aegypti* was colonized from larvae collected in 1998 in Surabaya and has been maintained for more than 300 generations.

RESULTS

The larvae of the laboratory strain have a mortality of 86% at a temephos dose of 0.012 mg/l. All field strains of *Ae.*

Table 1
Susceptibility of Surabaya strains of *Ae. aegypti* to temephos.

District of Surabaya	Subdistrict	% Mortality[a]		LT_{50} (95% CL)[c]	Resistance ratio[d]
		0.012 mg/l[b]	1 mg/l[b]		
Center	Tegalsari	52[g]	90[f]	101 (78.7-134.5)	2.8
	Simokerto	38[g]	87[f]	107 (79.6-151.7)	3.0
West	Sukomanunggal	51[g]	93[f]	88 (69.2-118.5)	2.5
	Tandes	39[g]	82[f]	142 (101.5-212.2)	4.0
North	Kenjeran	60[g]	96[f]	76 (58.0-102.2)	2.2
	Bulak	59[g]	92[f]	92 (73.6-118.6)	2.6
East	Tambaksari	25[g]	75[g]	200 (140.3-317.3)	5.6
	Gubeng	26[g]	76[g]	198 (140.8-340.6)	5.6
	Sukolilo	53[g]	92[f]	91 (69.4-125.0)	2.6
	Mulyorejo	52[g]	89[f]	107 (81.7-144.8)	2.3
South	Dukuh Kupang	50[g]	90[f]	100 (75.5-138.3)	2.8
	Sawahan	22[g]	72[g]	302 (223.1-444.0)	8.5
(Laboratory Strain)		86[f]	100[e]	35.6 (31.7-39.7)	-

[a]Susceptibility status based on percent mortality: [e]susceptible (≥98%), [f]tolerant or moderately resistant (80-98%), and [g]resistant (< 80%).
[b]Temephos dosage used for susceptibility testing using WHO methods.
[c]The lethal time required to kill 50% of larvae. CL, confidence limit.
[d]Mosquitoes were regarded as resistant when the resistance ratio was >1, while mosquitoes with a ratio of ≤1 were considered susceptible.

aegypti were resistant to temephos using WHO criteria. Five strains has a mortality rate of <50%. The Kenjeran and Bulak strains, collected in North Surabaya, had mortality rates of 60% and 59%, respectively, indicating higher susceptibility rates than the strains isolated from other areas in Surabaya. Even a temephos dose of 1 mg/l, larvae collected from three subdistricts (Tambaksari, Gubeng and Sawahan) have mortality rates of less than 80%. In another susceptibility test, field strains of larvae had LT_{50} values ranging from 76 to 302 minutes, longer than those obtained with the laboratory strains (36 minutes). This suggests all the *Ae. aegypti* strains obtained from the 12 different areas in Surabaya were resistant to temephos. The highest resistance ratio was in larvae from Sawahan (8.5) and the lowest was in larvae from Kenjeran (2.2). The results obtained using the WHO susceptibility test are consistent with those obtained by another susceptibility test based on LT_{50} values.

DISCUSSION

At a temephos dosage of 0.012 mg/l, as recommended by the WHO (WHO, 1992), the mortality rates ranged from 22% to 60% among Surabaya strains, indicating resistance to temephos. Similar findings have been reported from many countries (Brown, 1986; Rawlins, 1998; de Carvalho *et al*, 2004; Biber *et al*, 2006, Chen *et al*, 2005). The susceptibility test based on LT_{50} values also suggested incipient resistance in the Surabaya strains, indicating the emergence of resistance to temephos among *Ae. aegypti* mosquitoes from Surabaya. A present study of temephos resistance in this area during 2005 (Ahmad *et al*, 2006), showed the resistance level of *Ae. aegypti* to temephos in Surabaya was low (resistance ratio of 3.1). A low level of resistance was detected in this study; mortality rates were >80% in the subdistricts of Tegalsari, Simokerto, Sukomanunggal, Tandes, Kenjeran, Bulak, Sukolilo, Mulyorejo and Dukuh Kupang, indicating temephos at 1 mg/l was effective against field strains. However, in the present study carried out in 2009-2010, mosquitoes collected from three subdistricts (Tambaksari, Gubeng and Sawahan) has mortality rates <80%. Temephos may become ineffective at a dosage of 1 mg/l, as reported in the Caribbean (Georghiou *et al*, 1987). The presence of resistance to temephos among local mosquitoes may be because this insecticide has been widely used for controlling *Ae. aegypti* since 1970. Lee *et al* (1998) reported other factors, such as the migration of mosquitoes from different areas, may also influence susceptibility in the field population. How rapidly an insecticide becomes ineffective depends on selection pressure for resistance, *ie*, how long, how often and how much of the insecticide is used (Hudson, 1983). Furthermore, cross resistance may evolve as a result of the use of insecticides and an alternative method may then be required for effective vector control.

ACKNOWLEDGEMENTS

We would like to thank the students from the Environmental Health Academy (Surabaya, Indonesia) for collecting the mosquito larvae. This work was supported in part by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

REFERENCES

Ahmad I, Astari S, Raharjo B,Tan M, Munif A. Resistance of *Aedes aegypti* from three provinces in Indonesia, to pyrethroid and organophosphate insecticides. Bandung, Indonesia: International Conference on Mathematics and Natural Sciences (ICMNS). 29-30 November 2006 : 226-9.

Biber PA, Duenas JR, Almeida Fl, Gardenal CN, Almirón WR. Laboratory evaluation of susceptibility of natural subpopulations of *Aedes aegypti* larvae to temephos. *J Am Mosq Control Assoc* 2006; 22: 408-11.

Brown AWA. Insecticide resistance in mosquitoes: a pragmatic review. *J Am Mosq Control Assoc* 1986; 2: 123-40.

de Carvalho MSL, Caldas ED, Degallier N, *et al*. Susceptibility of *Aedes aegypti* larvae to the insecticide temephos in the Federal District, Brazil. *Rev Panam Saud Publica* 2004; 38: 623-9.

Chen CD, Nazni WA, Lee HL, Sofian Azirun M. Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Centre and Selangor State, Malaysia. *Trop Biomed* 2005; 22: 207-16.

Georghiou GP, Wirth M, Tran H, Saume F, Knudsen AB. Potential for organophosphate resistance in *Aedes aegypti* (Diptera: Culicidae) in the Caribbean area and neighboring countries. *J Med Entomol* 1987; 24: 290-4.

Hudson JE. Susceptibility of *Aedes aegypti* and *Culex quinquefasciatus* to insecticide in Paramoribo, Surinam, 1979-1981 and experimental selection for resistance. *Cah ORSTOM Ser Entomol Med Parasitol* 1983; 21: 275-9.

Lee HL, Nor Asikin, Nazni WA, Sallehuddin S. Temporal variations of insecticide susceptibility status of field-collected *Aedes albopictus* Skuse in Malaysia. *Trop Biomed* 1998; 15: 43-50.

Ministry of Health (MOH) Indonesia. Guidance book for DHF prevention and control in Indonesia Package B. Jakarta: MOH, 1999: 1-5.

Rawlins SC. Spatial distribution of insecticide resistance in Caribbean population of *Aedes aegypti* and its significance. *Rev Panam Saud Publica* 1998; 4: 243-51.

Suroso T. A review of dengue hemorrhagic fever and its control in Indonesia. Yogyakarta, Indonesia: Seminar on Recent Advance in Molecular Diagnostic, 1997.

World Health Organization (WHO). Instructions for determining the susceptibility of resistance of mosquito larvae to insecticides. *WHO/VBC/81.807*. 1981.

World Health Organization (WHO). Vector resistance to pesticides: Fifteenth report of the WHO Expert Committee on vector biology and control. *WHO Tech Rep Ser* 1992; 818: 56.