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Preliminary dengue vector surveillance in the Sunda Islands, Indonesia: Interchange of breeding habitat preferences of *Aedes aegypti* and *Aedes albopictus*

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Abstract. Ovitrap surveillance was conducted to determine the infestation patterns of dengue vectors in fourteen study sites across eight provinces located in the Sunda Islands, Indonesia. High ovitrap indices up to 70% and 90% were obtained from indoor and outdoor areas, respectively. Mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.13 to 14.50 and 0.10 to 18.60, respectively. Mixed infestation (<10%) and interchange of breeding habitat preferences of *Ae. albopictus* and *Ae. aegypti* were also observed in the present study.

INTRODUCTION

Dengue is a mosquito-borne viral disease that is currently considered as an important public health problem due to its rapid spread around the world. There is a dramatic increase of dengue cases globally and about half of the world's population is now at risk (WHO, 2014). Particularly, Indonesia has reported a total of 126,675 dengue cases with 1,229 deaths in 2015 (Ministry of Health Republic of Indonesia, 2016).

Aedes aegypti and *Aedes albopictus* have been reported as dengue vectors in the tropical and subtropical regions (WHO, 2014). *Ae. aegypti* is usually found in densely populated neighbourhoods and urbanized areas, whereas *Ae. albopictus* is recognized as rural mosquito and commonly breeds outdoors in the natural habitats such as tree holes (Mohiddin *et al.*, 2015).

Vector surveillance is an important element of vector control programs. The information obtained from the surveillance is important in determining the vector density, larval habitats, distribution of the mosquitoes and could be used qualitatively and quantitatively to predict the occurrence of disease outbreaks (Kusriastuti & Sutomo, 2005; Norzahira *et al.*, 2011). The most common method to monitor *Aedes* population is through ovitrap surveillance (Lau *et al.*, 2013). Other than simple and convenient, ovitrap is fast, sensitive and cost-effective to determine the presence of egg laying females of *Aedes* mosquitoes

(Norzahira *et al.*, 2011; Wan-Norafikah *et al.*, 2011).

Numerous dengue vector surveillance studies have been conducted but little has been focused on the vector abundance in Indonesia (Nusa *et al.*, 2014; Fahri *et al.*, 2013, Wijayanti *et al.*, 2016; Martini *et al.*, 2017). It is hypothesized that different geographic regions may have different infestation patterns of dengue vector. Thus, the aim of this study is to provide an updated information on the infestation pattern of *Aedes* species across seven provinces in Indonesia.

MATERIALS AND METHODS

Study sites

A cross-sectional ovitrap surveillance was conducted in September to November 2017 at 14 study sites across seven provinces in Indonesia. The geographical description of the study sites is given in Table 1. Dry weather with light rain was observed throughout the sampling periods.

Ovitrap surveillance

Preparation of ovitrap was performed according to Lee (1992). Ovitrap used was a 300ml plastic container with base diameter of 6.5cm, 9.0 cm in height and 7.8cm opening measured in diameter. A layer of black oil paint was sprayed and coated on the outer wall of the container. An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally into each ovitrap. Tap water was filled into each ovitrap to a level of 5.5 cm and the ovitraps were placed randomly not less than 10% of the total houses in each study site. Ovitraps were placed on the ground within 25 meters apart from each other. In this study, interior of the house is referred "indoor", while outside of the house but confined to the immediate vicinity of the house (i.e. car porch and corridor under the eave) is referred "outdoor" (Wan Norafikah *et al.*, 2011). The houses were selected following the guideline as mentioned by Rozilawati *et al.*, 2007 and upon permission from house owners;

i) the traps were located near other potential breeding containers
ii) the traps were in partial or total shade, not exposed to direct sunlight

Larvae identification

Ovitraps were collected after five days and brought back to laboratory. The contents were poured into individual plastic containers, together with the paddle. Each container was filled with fresh water and the larvae were allowed to grow under the laboratory conditions at 25°C and 65±20% relative humidity. To avoid oviposition by other mosquitoes in vicinity, the containers were kept covered. A small amount (0.01g) of powdered beef liver was added into each container as larval food. The hatched larvae were subsequently counted and identified at 3rd instar and the numbers of larvae were recorded individually for each positive ovitrap (Chen *et al.*, 2005).

Data analysis

The percentage of positive ovitraps to the total number of recovered ovitraps was determined for each ovitrap surveillance site to obtain the Ovitrap Index (OI). Mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae per total number of recovered ovitrap were also determined. Paired t-test was performed using SPSS (Version 25) to test if the mean numbers of larvae per ovitrap differ for the two species in both indoor and outdoor areas. All levels of statistical significance were determined at $p \leq 0.05$.

RESULTS

A total of 385 ovitraps were placed indoor and outdoor randomly across all study sites, and a total of 2,431 larvae were examined, of which 66.52% were *Ae. albopictus* and 33.48% were *Ae. aegypti*.

The indoor OIs ranged from 0.00 to 70.00%, with the highest OI recorded in Sanur (Bali). The mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.27 to 13.35 and 0.29 to 9.00,

Table 1. Geographical description of study sites in Indonesia

Sunda Island	Island	Province	Regency	Study site	Coordinates	Landscape
Greater Sunda Islands	Java	West Java	Kuningan	Kuningan	S 6°13'5.260" E 106°50'15.936"	Sub-urban
	Sumatra	West Sumatra	Padang	Air Tawar Barat	S 0°53'48.260" E 100°20'45.265"	Sub-urban
		Aceh	Aceh	Banda Aceh	N 5°34'27.170" E 95°22'6.517"	Sub-urban
	Borneo	East Kalimantan	Samarinda	Sidodadi	S 0°28'41.646" E 117°08'46.441"	Sub-urban
			Paser	Long Ikis	S 1°36'15.486" E 116°10'1.292"	Rural
		West Kalimantan	Pontianak	Bangka Belitung Laut	S 0°3'31.967" E 109°21'19.322"	Sub-urban
Lesser Sunda Islands	Bali	Bali	Denpasar	Sanur	S 8°41'10.254" E 115°15'23.634"	Sub-urban
	Lombok	West Nusa Tenggara	Mataram	Ampenan	S 8°34'13.911" E 116°05'08.575"	Sub-urban
				Pagesangan	S 8°36'2.666" E 116°06'07.080"	Sub-urban
	Sumbawa		Dompu	Bada	S 8°32'20.878" E 118°27'28.799"	Sub-urban
	Flores	East Nusa Tenggara	Manggarai Barat	Labuan Bajo	S 8°29'34.269" E 119°52'40.889"	Sub-urban
	Sumba		Southwest Sumba	Tambolaka	S 9°25'50.416" E 119°14'18.636"	Sub-urban
			East Sumba	Waingapu	S 9°39'49.331" E 120°16'17.321"	Sub-urban
	Timor		South Central Timor	Soe	S 9°51'33.538" E 124°15'44.345"	Sub-urban

spectively. Generally, mean numbers of *Ae. aegypti* and *Ae. albopictus* populations obtained from indoor (10 out of 13 sites) were not significantly different ($p>0.05$). Only the populations of *Ae. aegypti* from Air Tawar Barat (West Sumatra) and Sanur (Bali) were found significantly dominant in indoor compared to *Ae. albopictus* ($p<0.05$). On the other hand, population of *Ae. albopictus* from Labuan Bajo (East Nusa Tenggara) was significantly higher than *Ae. aegypti* ($p<0.05$). No *Aedes* larvae were found in ovitraps placed indoor in Banda Aceh (North Sumatra) and Pagesangan (West Nusa Tenggara). *Aedes albopictus* was absent in indoor areas in eight study sites (Table 2).

The outdoor OIs ranged from 0.00 to 90.00%, with the highest OI recorded in Labuan Bajo (East Nusa Tenggara). Mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.13 to 14.20 and 0.10 to 18.60, respectively. Although *Ae. albopictus* was more prevalent than *Ae. aegypti* in majority sites (8 out of 14), only six study sites were found significantly dominant in outdoor populations ($p<0.05$). *Ae. aegypti* populations from Kuningan (West Java) and Sidodadi (East Kalimantan) were significantly dominant in outdoor compared to *Ae. albopictus* ($p<0.05$). No *Aedes* larvae were found in ovitraps placed outdoor areas in Bada (West Nusa Tenggara) and Soe (East Nusa Tenggara) (Table 3).

Overall, the mean numbers of *Ae. aegypti* larvae per ovitrap were 3.17 ± 0.68 for indoor and 1.19 ± 0.51 for outdoor. On the other hand, the mean numbers of *Ae. albopictus* larvae per ovitrap were 1.19 ± 0.29 for indoor and 6.78 ± 0.89 for outdoors. Both *Ae. aegypti* and *Ae. albopictus* differ significantly in indoor and outdoor ($p< 0.01$). Nonetheless, only six study sites (i.e., Air Tawar Barat, Sanur, Banda Aceh, Sidodadi, Sanur and Labuan) showed significant difference in mean larvae numbers for *Ae. aegypti* and *Ae. albopictus* in indoor and outdoor areas ($p <0.05$) (Table 4).

Mixed infestation of *Ae. aegypti* and *Ae. albopictus* was also observed in this study. Only 5.4% ovitraps (10 out of 185 ovitraps) had mixed infestations in indoor areas, with predomination of *Ae. aegypti* larvae in Sanur by 5.36 fold, and *Ae. albopictus* larvae in Labuan Bajo by 1.26 fold. On the other hand, co-occurrence of both *Aedes* species was also found in 8.5% ovitraps (17 out of 200 ovitraps) in outdoor across seven study sites. *Ae. albopictus* and *Ae. aegypti* larvae were found dominating in mixed infestation ovitraps in five study sites by 7.91 – 119.67 folds, and two study sites by 6.50 – 9.67 folds, respectively.

DISCUSSION

Information on *Aedes* larval densities in terms of space and time for determining the major breeding sources as well as predicting dengue outbreaks can be obtained through ovitrap surveillance (Tham, 2000). In this study, more positive ovitraps were found outdoors rather than indoors in all study sites. Similar findings have also been reported by Wan-Norafikah *et al.* (2011) in Peninsular Malaysia.

Ae. aegypti can be mostly found exclusively inside houses and feeds mostly indoor (Rudnick, 1986). In the present study, *Ae. aegypti* was found dominant in indoors at 7 out of 13 study sites. This is consistent with recent studies conducted in Perak, Selangor, Kuala Lumpur and Penang, Malaysia where *Ae. aegypti* is still prefers to breed indoor (Ho *et al.*, 2014; Rozilawati *et al.*, 2015). In contrast, *Ae. albopictus* breeds in manmade and natural containers and mostly found in outdoor areas (Saleeza *et al.*, 2013). Likewise, *Ae. albopictus* was also commonly found at outdoor areas (8 out of 14 study sites). This finding is also consistent with the studies conducted in Selangor, Kuala Lumpur and Penang Island in Malaysia (Rozilawati *et al.*, 2015).

Interestingly, interchange of breeding habitat preferences in *Ae. aegypti* and *Ae. albopictus* was observed in four study sites. Previous studies elsewhere have also reported similar phenomenon: *Ae. albopictus* breeds indoors, and in contrast *Ae. aegypti* breeds outdoors (Syarifah *et al.*, 2008; Saleeza *et al.*, 2011; Wan-Norafikah *et al.*, 2011; Wan-Norafikah *et al.*, 2012). Based

Table 2. Ovitrap index (indoor), mean numbers of larvae and percentage of larval density recovered in Indonesia

Province	Regency	Study site	Number of ovitrap	Ovitrap Index (%)	Mean numbers (± SE)			Percentage of larval density (%)	
					Ae. aegypti	*Ae. albopictus*	t-test	*Ae. aegypti*	*Ae. albopictus*
West Java	Kuningan	Kuningan	15	6.67	0.27 ± 0.27	0.00 ± 0.00	$p = 0.334$ df = 14	100	0.00
West Sumatra	Padang	Air Tawar Barat	20	60.00	13.35 ± 4.42	0.00 ± 0.00	$p = 0.007^{*}$ df = 19	100	0.00
North Sumatra	Aceh	Banda Aceh	15	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
East Kalimantan	Samarinda	Sidodadi	7	14.29	0.29 ± 0.29	0.29 ± 0.29	$p = 0.356$ df = 6	50	50
West Kalimantan	Pontianak	Bangka Belitung Laut	10	10.00	1.10 ± 1.10	0.00 ± 0.00	$p = 0.343$ df = 9	100	0.00
Bali	Denpasar	Sanur	20	70.00	3.75 ± 1.09	0.70 ± 0.40	$p = 0.015^{*}$ df = 19	84.27	15.73
West Nusa Tenggara	Mataram	Ampenan	10	20.00	0.70 ± 0.70	2.10 ± 2.10	$p = 0.535$ df = 9	25.00	75.00
		Pagesangan	10	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
	Dompu	Bada	10	10.00	1.30 ± 1.30	0.00 ± 0.00	$p = 0.343$ df = 9	100	0.00
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	20	60.00	7.15 ± 2.74	9.00 ± 2.67	$p = 0.631$ df= 19	44.27	55.73
	Southwest Sumba	Tambolaka	15	60.00	0.33 ± 0.33	8.00 ± 3.63	$p = 0.033^{*}$ df = 14	3.96	96.39
	East Sumba	Waingapu	15	13.33	3.13 ± 2.86	0.00 ± 0.00	$p = 0.292$ df = 14	100	0.00
	South Central Timor	Soe	18	5.56	0.67 ± 0.67	0.00 ± 0.00	$p = 0.331$ df = 17	100	0.00

N/A: Not available.
*Statistically significant ($p \leq 0.05$).

Table 3. Ovitrap index (outdoor), mean numbers of larvae, and percentage of larval density recovered in Indonesia

Province	Regency	Study site	Number of ovitrap (n)	Ovitrap Index (%)	Mean numbers (±SE)			Percentage of larval density (%)	
					Ae. aegypti	*Ae. albopictus*	t-test	*Ae. aegypti*	*Ae. albopictus*
West Java	Kuningan	Kuningan	14	35.71	0.50 ± 0.19	0.00 ± 0.00	$p = 0.029^{*}$ df= 13	100	0.00
West Sumatra	Padang	Air Tawar Barat	20	85.00	0.15 ± 0.11	17.95 ± 3.52	$p = 0.001^{*}$ df= 19	0.83	99.17
North Sumatra	Aceh	Banda Aceh	15	80.00	0.00 ± 0.00	4.93 ± 1.75	$p = 0.014^{*}$ df= 14	0.00	100
East Kalimantan	Samarinda	Sidodadi	8	87.50	14.50 ± 3.54	1.50 ± 1.05	$p = 0.008^{*}$ df= 7	90.63	9.38
	Paser	Long Ikis	15	33.33	0.00 ± 0.00	3.53 ± 1.59	$p = 0.043^{*}$ df= 14	0.00	100
West Kalimantan	Pontianak	Bangka Belitung Laut	10	10.00	0.00 ± 0.00	0.10 ± 0.10	$p = 0.343$ df= 9	0.00	100
Bali	Denpasar	Sanur	20	75.00	0.50 ± 0.35	4.40 ± 1.52	$p = 0.033^{*}$ df= 19	10.20	89.80
West Nusa Tenggara	Mataram	Ampenan	10	60.00	0.60 ± 0.43	12.30 ± 5.79	$p = 0.074$ df= 9	4.65	95.35
		Pagesangan	10	40.00	1.30 ± 0.80	0.20 ± 0.20	$p = 0.213$ df= 9	86.67	13.33
	Dompu	Bada	10	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	20	90.00	2.35 ± 1.00	18.60 ± 3.24	$p = 0.001^{*}$ df= 19	11.22	88.78
	Southwest Sumba	Tambolaka	15	80.00	0.13 ± 0.09	15.27 ± 4.08	$p = 0.002^{*}$ df= 14	0.84	99.16
	East Sumba	Waingapu	15	20.00	1.20 ± 0.73	0.00 ± 0.00	$p = 0.123$ df= 14	100	0.00
	South Central Timor	Soe	18	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	

N/A: Not available.
*Statistically significant ($p \leq 0.05$).

Table 4. Comparison of *Aedes albopictus* and *Aedes aegypti* obtained from indoor and outdoor areas

Province	Regency	Study site	*Ae. albopictus*	*Ae. aegypti*
West Java	Kuningan	Kuningan	N/A	$p = 0.384$ df = (1,14)
West Sumatra	Padang	Air Tawar Barat	$p = 0.01$* df = (1,19)	$p = 0.007$* df = (1,19)
North Sumatra	Aceh	Banda Aceh	$p = 0.014$* df = (1,14)	N/A
East Kalimantan	Samarinda	Sidodadi	$p = 0.245$ df = (1,6)	$p = 0.014$* df = (1,6)
West Kalimantan	Pontianak	Bangka Belitung Laut	$p = 0.343$ df = (1,9)	$p = 0.343$ df = (1,9)
Bali	Denpasar	Sanur	$p = 0.03$* df = (1,19)	$p = 0.03$* df = (1,19)
West Nusa Tenggara	Mataram	Ampenan	$p = 0.137$ df = (1,19)	$p = 0.798$ df = (1,9)
		Pagesangan	$p = 0.343$ df = (1,9)	$p = 0.14$ df = (1,9)
	Dompu	Bada	N/A	$p = 0.343$ df = (1,9)
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	$p = 0.016$* df = (1,19)	$p = 0.076$ df =(1,19)
	Southwest Sumba	Tambolaka	$p = 0.210$ df = (1,19)	$p = 0.486$ df = (1,19)
	East Sumba	Waingapu	N/A	$p = 0.445$ df =(1,19)
	South Central Timor	Soe	N/A	$p = 0.331$ df = (1,17)

on the independent t-test, there is no significant difference between different landscapes with OI and mean numbers of larvae per ovitrap for *Ae. aegypti* and *Ae. albopictus*. However, study conducted by Braks *et al.* (2003) showed that *Ae. aegypti* was predominant in urbanized areas while *Ae. albopictus* were more abundant in rural areas, and co-occurrence of both species can be found in sub-urban areas.

Ae. albopictus and *Ae. aegypti* are sympatric species that occupy similar ecological niches (Wan-Norafikah *et al.*, 2012). Mixed infestation of both species was also found in both indoor and outdoor areas (7 out of 14 study sites) ranged from 11.76 to 66.67%. The present results were much higher than the mixed infestation rates reported in Malaysia (5 to 45%) (Chen *et al.*, 2006; Wan-Norafikah, 2011). Additionally, previous studies also showed the mixed infestation of *Aedes* with other genera of mosquitoes such as *Armigeres* spp. and *Culex quinquefasciatus* (Chen *et al.*, 2006; Lau *et al.*, 2017).

In conclusion, high ovitrap indices detected in the present study strongly suggest the risk of transmission of dengue viruses by both *Ae. aegypti* and *Ae. albopictus*. Mosquito breeding habitat source reduction must be implemented and public awareness of dengue should be inculcated to the community members and civilians through health education. With the participation and cooperation from the community, it is hoped that the populations of mosquitoes can be reduced, and thereby reducing the burden of dengue in this region.

Table 5. Mixed infestation of *Aedes* in ovitraps obtained from Indonesia

Regency	Study site	Number of recovered ovitraps	Total positive ovitraps	Ovitraps with mixed infestation	Percent positive ovitrap (%)			Ratio of *Ae. aegypti: Ae. albopictus* in mixed infestation
					Ae. aegypti	*Ae. albopictus*	Mixed infestation	
				Indoor				
Kuningan	Kuningan	15	1	0	100.00	0.00	0.00	N/A
Padang	Air Tawar Barat	20	12	0	100.00	0.00	0.00	N/A
Aceh	Banda Aceh	15	0	0	0.00	0.00	0.00	N/A
Samarinda	Sidodadi	7	1	0	100.00	0.00	0.00	N/A
Pontianak	Bangka Belitung Laut	10	1	0	100.00	0.00	0.00	N/A
Denpasar	Sanur	20	14	2	78.57	7.14	14.29	5.36 : 1.00
Mataram	Ampenan	10	2	0	50.00	50.00	0.00	N/A
	Pagesangan	10	0	0	0.00	0.00	0.00	N/A
Dompu	Bada	10	1	0	100.00	0.00	0.00	N/A
Manggarai Barat	Labuan Bajo	20	12	8	8.33	25.00	66.67	1.00 : 1.26
Southwest Sumba	Tambolaka	15	9	0	11.11	88.89	0.00	N/A
East Sumba	Waingapu	15	2	0	100.00	0.00	0.00	N/A
South Central Timor	Soe	18	1	0	100.00	0.00	0.00	N/A
				Outdoor				
Kuningan	Kuningan	14	5	0	100.00	0.00	0.00	N/A
Padang	Air Tawar Barat	20	17	2	0.00	88.24	11.76	1.00 : 119.67
Aceh	Banda Aceh	15	12	0	0.00	0.00	0.00	N/A
Samarinda	Sidodadi	8	7	2	71.43	0.00	28.57	9.67 : 1.00
Paser	Long Ikis	15	5	0	0.00	100.00	0.00	N/A
Pontianak	Bangka Belitung Laut	10	1	0	0.00	100.00	0.00	N/A
Denpasar	Sanur	20	15	2	0.00	86.67	13.33	1.00 : 8.80
Mataram	Ampenan	10	6	2	0.00	66.67	33.33	1.00 : 20.50
	Pagesangan	10	4	1	75.00	0.00	25.00	6.50 : 1.00
Dompu	Bada	10	0	0	0.00	0.00	0.00	N/A
Manggarai Barat	Labuan Bajo	20	18	6	5.56	61.11	33.33	1.00 : 7.91
Southwest Sumba	Tambolaka	15	12	2	0.00	83.33	16.67	1.00 : 18.00
East Sumba	Waingapu	15	3	0	100.00	0.00	0.00	N/A
South Central Timor	Soe	18	0	0	0.00	0.00	0.00	N/A

N/A: Not available

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