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Short Communication

Detection and Serotyping of Dengue Viruses in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Collected in Surabaya, Indonesia from 2008 to 2015

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SUMMARY: *Aedes aegypti* and *Aedes albopictus* are the primary and secondary vectors, respectively, of dengue, the most important arboviral disease in the world. The aim of this study was to detect and serotype dengue viruses (DENV) in the vectors *Ae. aegypti* and *Ae. albopictus* in Surabaya, Indonesia. Between 2008 and 2015, 16,605 *Aedes* mosquitoes were collected in 15 sub-districts of Surabaya. *Ae. aegypti* was dominant (90.9%), whereas few *Ae. albopictus* were collected (9.1%). A total of 330 pools of adult *Aedes* mosquitoes were subjected to the serotyping of DENV by RT-PCR. DENV-1 (52.3%) was the most frequently detected serotype, followed by DENV-2 (40.3%), DENV-4 (4.6%), and DENV-3 (2.8%). The average minimum infection rate for *Ae. aegypti* in various sub-districts of Surabaya was 7.2 per 1,000 mosquitoes, while that for *Ae. albopictus* was 0.7 per 1,000 mosquitoes. The results showed that the predominantly circulating DENV serotype in mosquitoes continuously shifted from DENV-2 (2008) to DENV-1 (2009–2012), to DENV-2 again (2013–2014), and then back to DENV-1 (2015). The circulating DENV serotypes in mosquitoes were generally consistent with those in humans. Therefore, the surveillance of infected mosquitoes with DENV might provide an early warning sign for the risk of future dengue outbreaks.

Dengue fever and dengue hemorrhagic fever, or dengue and severe dengue, are globally important arboviral diseases with a 30-fold increase being reported in the number of human cases in the last 50 years (1,2). Endemicity for dengue is also expanding to dengue-free countries, including Japan (1,3). The estimated number of annual dengue cases ranges between 50 and 100 million, with approximately 2.5 billion individuals being at risk, 75% of whom are located in the South East Asian and the Pacific regions (1).

Dengue virus (DENV) comprises 4 genetically and antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). DENV is transmitted by mosquitoes belonging to the genus *Aedes*, particularly *Ae. aegypti* and *Ae. albopictus*. The dynamics of dengue outbreaks is complex and characterized by the frequent replacement of virus lineages and serotypes (4). The exact mechanism for this replacement is still unknown; however, purifying selection by host immune responses (5), lineage bottlenecks, and environmental impacts on the mosquito populations were found to be the key factors involved in the replacement of viral lineages and serotypes (6).

Indonesia is a tropical country that is hyperendemic to dengue, wherein all the 4 DENV serotypes have been circulating. Dengue cases have been reported in all the 34 provinces in Indonesia throughout the year, and outbreaks occur periodically (7). Surabaya is the second largest city of Indonesia. Previous studies on the isolation of DENVs from human sera indicated that serotype shifts from DENV-2 to DENV-1 occurred in 2008, followed by the replacement from DENV-1 to DENV-2 in 2013 in Surabaya (8,9). However, epidemiological surveys of DENV, particularly, the detection of DENVs in *Aedes* mosquitoes, are limited in Indonesia. Therefore, in this study, we performed the detection and serotyping of DENV in *Aedes* mosquitoes collected from the dengue endemic sub-districts of Surabaya, Indonesia.

Adult mosquitoes were collected between 2008 and 2015 from 15 sub-districts (Tegalsari, Simokerto, Sukomanunggal, Tandes, Asemrowo, Kenjeran, Bulak, Tambaksari, Gubeng, Sukolilo, Rungkut, Mulyorejo, Dukuh Pakis, Sawahan, and Wonocolo) of Surabaya, Indonesia (17°15′55″S; 112°44′33″E), which reported confirmed dengue cases. *Aedes* spp. were collected

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from the houses of the residents diagnosed with dengue fever and from another 5 residences (neighboring houses) adjacent to the dengue patient house. Adult mosquitoes were captured indoors and outdoors using yeast-generated CO_2 traps and sweep nets. The collected mosquitoes were freeze-killed at −30°C and sorted by species, date, and location. Male and female mosquitoes were distinguished based on the morphological differences of their antennae. All adult female *Aedes* mosquitoes were pooled (primarily 30–50 individuals/pool) and then subjected to RNA extraction using the QIAamp Viral Mini Spin Kit (Qiagen, Hilden, Germany). The presence of viral RNA was confirmed by RT-PCR (10). Briefly, viral RNA was reverse transcribed to cDNA using the SuperScript III First-Stand Synthesis Kit (Invitrogen, Carlsbad, CA, USA) with the reverse primer, D2 (5'-TTGCACCAACAGTCAATGTCTTCAGGTTC-3'). DENV-1, DENV-2, DENV-3, and DENV-4 genomes were amplified by a multiplex PCR using rTaq (Toyobo, Osaka, Japan), along with the sense primer D1 (5'-TCAATATGCTGAAACGCGCGAGAAACCG-3') and the serotype specific reverse primers (TS1: 5'-CGTCTCAGTGATCCGGGGG-3'; TS2: 5'-CGCCACAAGGGCCATGAACAG-3'; TS3: 5'-TAACATCATCATGAGACAGAGC-3'; and TS4: 5'-CTCTGTTGTCTTAAACAAGAGA-3') to generate 482, 118, 290, and 392-bp fragments, respectively. The DENV serotype was determined by the size of the amplified fragment. The minimum infection rate (MIR) was estimated to compare the virus infection rates in the mosquitoes collected from different sampling points. MIR was calculated for each sub-district according to the standard formula: (the number of DENV-positive mosquito pools/the total number of mosquitoes tested) × 1,000 (11).

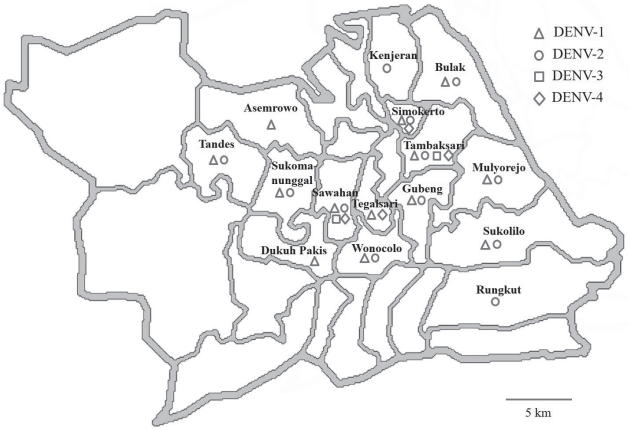

Fig. 1. Geographic distribution of DENV detected from *Aedes* mosquitoes in sub-districts of Surabaya between 2008 and 2015.

Between January 2008 and December 2015, 16,605 female *Aedes* mosquitoes were collected from the 15 sub-districts of Surabaya, encompassing all the geographical zones (Fig. 1). *Ae. aegypti* was the dominant species (90.9%), whereas few *Ae. albopictus* were collected (9.1%), indicating that *Ae. aegypti* was the principal dengue vector in Surabaya. Due to its close association with humans, this species has played a major role in the worldwide emergence of dengue for the last 50 years (1).

Two hundred ninety six pools and 34 pools were prepared from 15,099 *Ae. aegypti* and 1,506 *Ae. albopictus*, respectively, collected from the sample sites (Table 1). Of the 296 *Ae. aegypti* pools assayed, 109 (36.8%, 109/296) showed RT-PCR positivity; wherein 57 pools (52.3%, 57/109) were positive for DENV-1, 44 pools (40.4%, 44/109) were positive for DENV-2, 3 pools (2.8%, 3/109) were positive for DENV-3, and 5 pools (4.6%, 5/109) were positive for DENV-4. Of the 34 *Ae. albopictus* pools assayed, only 1 was positive for DENV-1. DENV-1 and DENV-2 were the most com-

Table 1. Identification of DENV serotypes detected from *Aedes* spp. in Surabaya

Region	Sub-distinct	*Ae. aegypti*			*Ae. albopictus*			Detected DENV serotype[1)]	
		Female mosquito (total pool)	Positive pool	MIR[2)]	Female mosquito (total pool)	Positive pool	MIR	*Ae. aegypti* (positive pool)	*Ae. albopictus* (positive pool)
Central	Tegalsari	856 (17)	8	9.3	0	0	0	D1 (6), D4 (2)	–
	Simokerto	978 (19)	6	6.1	0	0	0	D1 (3), D2 (2), D4 (1)	–
West	Sukomanunggal	1,038 (20)	5	4.8	382 (7)	0	0	D1(2), D2 (3)	–
	Tandes	850 (17)	4	4.7	0	0	0	D1 (2), D2 (2)	–
	Asemrowo	622 (12)	2	3.2	0	0	0	D1 (2)	–
North	Kenjeran	826 (16)	1	1.2	80 (4)	0	0	D2 (1)	–
	Bulak	817 (16)	6	7.3	0	0	0	D1 (3), D2 (3)	–
East	Tambaksari	1,643 (32)	19	11.6	480 (9)	0	0	D1 (9), D2 (8), D3 (1), D4 (1)	–
	Gubeng	1,405 (28)	15	10.6	0	0	0	D1 (7), D2 (8)	–
	Sukolilo	636 (12)	4	6.3	0	0	0	D1 (3), D2 (1)	–
	Rungkut	453 (9)	2	4.4	20 (1)	0	–	D2 (2)	–
	Mulyorejo	980 (19)	6	6.1	240 (6)	0	–	D1 (4), D2 (2)	–
South	Dukuh Pakis	968 (19)	3	3.0	124 (3)	0	–	D1 (3)	–
	Sawahan	2,163 (43)	24	11.0	180 (4)	1	5.5	D1 (10), D2 (11), D3 (2), D4 (1)	D1 (1)
	Wonocolo	864 (17)	4	4.6	0	0	0	D1 (3), D2 (1)	–
	Total	15,099 (296)	109	7.2	1,506 (34)	1	0.7	D1 (57), D2 (44), D3 (3), D4 (5)	D1 (1)

[1)]: No co-infection case was detected. DENV-1, -2, -3, and -4 were abbreviated by D1, D2, D3, and D4, respectively.
[2)]: Minimum infection rate (MIR) = (No. of positive pools/No. of mosquitoes) × 1,000.

monly detected serotypes in most of the sub-districts, although the sampling points were located far from each other (Fig. 1). *Aedes* mosquitoes do not migrate over large areas and are generally limited to a radius of 150 m from their breeding site (12). It was revealed that human movement, instead of mosquito movement might have contributed to the dissemination of DENV in Surabaya, as reported in Rio de Janeiro, Brazil (13).

The MIRs for *Ae. aegypti* and *Ae. albopictus* in each sub-district area are shown in Table 1. The average MIR for *Ae. aegypti* in the various sub-districts of Surabaya was 7.2 per 1,000 mosquitoes (ranged between 1.2 and 11.6 per 1,000 mosquitoes), whereas it was 0.7 per 1,000 mosquitoes (ranged between 0 and 5.5 per 1,000 mosquitoes) for *Ae. albopictus*. These MIR values were lower than that of the previous findings, such as those from Venezuela (16.0 per 1,000 mosquitoes) (14) and Singapore (57.6 per 1,000 mosquitoes) (15). Many factors might have contributed to the low MIRs obtained in this study. Mosquito collections were conducted late (1–2 weeks after the outbreak), and fogging insecticide might have also reduced the number of infected mosquitoes collected during this study.

Chronological detection data showed the dominance of DENV-2 in 2008, followed by DENV-1 dominance between 2009 and 2012 (Fig. 2). In 2013, although all DENV serotypes were detected, DENV-2 was dominant, indicating a serotype shift, which also continued till 2014. In 2015, DENV-1 was dominant again, despite the detection of all the 4 DENV serotypes, indicating another serotype shift. We have previously reported the sequential changes in the predominant DENV serotype isolated from the dengue patient sera in this area; i.e., from DENV-2 to DENV-1 in November 2008, and from DENV-1 to DENV-2 in July 2013 (8,9). Taken together, the predominant DENV serotypes detected in the mosquitoes were generally consistent with those detected in human sera. A previous study reported that the DENV serotype shift was associated with a dengue outbreak (9). The surveillance of infected mosquitoes with DENV may provide an early warning sign for assessing the risk of a dengue outbreak.

A phylogenetic analysis might have provided interesting insights, however, it was not conducted in the present study due to the failure of PCR. Phylogenetic analysis should be conducted in future to compare the DENVs circulating in the different sub-districts as well as in the mosquitoes and humans.

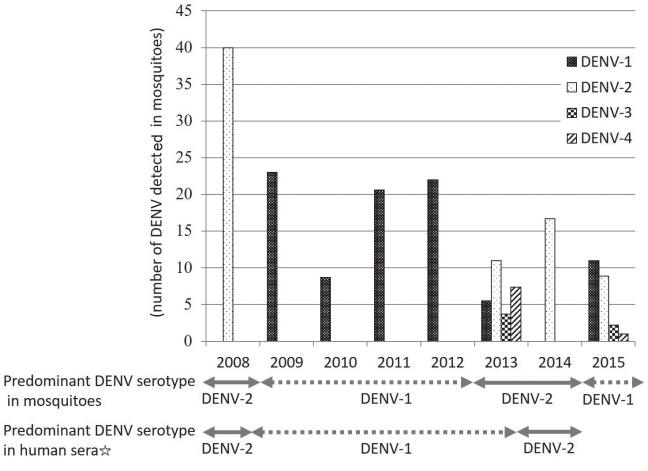

Fig. 2. Chronological data on DENV detected from *Aedes* mosquitoes collected in Surabaya between 2008 and 2015. ☆ The information of predominant DENV serotype in human sera was retrieved from the previous studies (8,9).

In conclusion, this study showed the sequential shifts of the predominant DENV serotype in mosquitoes, which were generally consistent with that in human sera. Monitoring of the virus circulation in mosquitoes will enable us to predict the risk of a dengue outbreak, and, will hence contribute to identifying the locations, which will require prevention and control measures.

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Conflict of interest None to declare.

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