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## Dengue vector surveillance using vector indices and ovitraps in Sujung village, Banten, Indonesia

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### Abstract

Dengue vector surveillance is one of important part of vector control. The aim of this study was to determine an area at risk for dengue transmission in Sujung Village. In 100 house inspections, sampling for larval and pupal stages was done indoors and outdoors. A total of 200 ovitraps (20 ovitraps/ house) were placed indoors and outdoors of inspected houses for 5 days. To detect dengue virus in female *Ae. aegypti* was used Qiagen kits. The house index, container index, Breteau index were 28%, 20.7%, and 28.0% respectively. There was a statistically significant strong negative correlation between number of water-holding containers and number of larval positive containers ( $R = -0.66$ ,  $p$  value = 0.000,  $p < 0.05$ ). Of 200 ovitraps, 2.5% (5/200) positive ovitraps were found. No dengue virus was detected in 35 female *Ae. aegypti* mosquitoes. The Sujung village was an area at risk of dengue transmission.

**Keywords:** surveillance, aedes aegypti, vector indices, sujung village, ovitrap

### 1. Introduction

*Aedes aegypti* is known as yellow fever mosquito that transmits a dengue disease, vector-borne diseases and the dengue disease remains public health problem in tropical and sub tropical countries<sup>[1, 2]</sup>. The incidence of dengue extremely increases worldwide in recent decades. Many dengue cases are underreported and misclassified. Approximately 390 million dengue infections per year in worldwide were diagnosed and as many as 96 million of 390 infected people exhibited clinical signs and severity of disease<sup>[3]</sup>. In addition, it is estimated that 3.9 billion people of 128 countries live in areas at risk of dengue transmission<sup>[4]</sup>.

*Ae. aegypti* belongs to Diptera ordo, which employs both natural and artificial habitats to continue their life cycle. *Ae. aegypti* can be adaptive to urban environments especially man-made containers or artificial containers such as vases, water tanks, care tyres, buckets and other artificial containers<sup>[5, 6]</sup>. The female *Ae. aegypti* mosquitoes lay their eggs on wet walls of containers with water. Within several days, the eggs develop into larvae. Then, the larvae can grow to fourth instars after they feed bacteria, fungi and others. Afterwards, the fourth instars develop into pupae in as little as 5 days. Finally, the newly formed adult emerges from the water after breaking the pupal skin in 2-3 days. This completely life cycle of *Ae. aegypti* take approximately 8-10 days at room temperature, depending on the level of feeding<sup>[7]</sup>.

The high density of *Ae. aegypti* mosquitoes in an area can induce the risk of dengue outbreak. To predict the risk of dengue outbreak employs vector surveillance based on entomological parameters<sup>[8]</sup>. This surveillance is important in determining factors related to dengue transmission, in order to prioritize areas and seasons for vector control<sup>[9]</sup>. The most used indicators for vector surveillance are house index (HI, percentage of houses infested with larvae and/or pupae), container index (CI, percentage of water-holding containers infested with larvae or pupae), Breteau index (BI, number of positive containers per 100 houses inspected), pupal index, and ovitrap indexes<sup>[8, 9]</sup>.

Sujung village is located in Banten province, West Java, Indonesia. Generally, sanitation of village is poor and many man-made breeding places around their house due to the problem of clean water. During the rainy season, many inhabitants collect rain water placed in these plastic buckets to hold the water. All of the households have the water-holding containers in their bathrooms. In this village, dengue control program from local government may be conducted irregularly. Furthermore, the community of the village may not conduct any vector surveillance.

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Therefore, in the Sujung village should be conducted the vector surveillance. The aim of this study was to evaluate the dengue vector surveillance using vector indices and ovitraps to determine an area at risk for dengue transmission in the Sujung Village.

## 2. Materials and Methods

### 2.1 Study area

The study was conducted from May to August of 2018, in Sujung village, located in Subdistrict Tirtayasa, District Serang, Banten Province, Indonesia. The width of Sujung village was 978.001 ha and the most of this area was rice fields. There were two seasons, dry and rainy seasons. The number of inhabitants was 5272 (2541 male and 2731 female). All of the inhabitants were Muslim (100%). Most of them were farmers. This village was divided into 10 neighborhood units or blocks. Each unit had a chairman.

### 2.2 Vector surveillance

A vector survey was carried out in 10 neighborhood units of Sujung village. Natural and artificial water-holding containers were visually inspected at each of 100 houses (10 houses/neighborhood) for the presence of mosquito larvae and pupae [10]. Each container was recorded on vector survey data sheets to note container type, indoor or outdoor, number of container, and water type. Immature stages, larvae and pupae, were identified in each container as follows: positive (+), indicating the presence of larval and/or pupal stages in a container and negative (-), indicating that no larval and/or pupal stages were found. Those larvae and pupae that were found were put into a plastic bottle/container using a dropper [11, 12]. Afterward, the larvae and pupae are carefully reared in the lab. After several days, the pupae matured into mosquitos whose species were subsequently identified.

### 2.3 Larval identification

Larvae collected from the field were identified to determine the species using the light microscope at the laboratory of Department of Parasitology, Faculty of Medicine, the University of Indonesia. The larval identification was described previously [12]. Briefly, Larvae were divided into three main parts: the head, thorax, and the abdomen. All three parts were observed to identify the setae, the segment VIII, the siphon, and the anal segment or the segment X, which resembles the parts and segments of an *Aedes* mosquito larvae. All larvae collected were identified, and only *Aedes* mosquito larvae counted, whereas larvae from other species were excluded.

### 2.4 Ovitrap

This study used ovitraps, no chemical or no hay in the ovitraps. White plastic glasses 200 mL in volume were used as ovitraps. A piece of filter paper (15 cm in length and 5 cm in width) was attached and 120 mL clean water was added to each glass. A total of 100 non-attractant ovitraps (two ovitraps/house) were placed at each household, one indoors and one outdoors. The ovitraps were placed outdoors, protected from rain and direct sunlight and out of reach of children and pets. After 1 week (5-7 days), the filter papers were collected and the eggs were transported to the laboratory for counting and species identification following hatching.

The percentage of positive ovitrap was calculated as follows: positive ovitrap/number of ovitrap x 100%.

## 2.5 Detection of dengue virus

The detection of dengue virus among female *Ae. aegypti* mosquitoes conducted at laboratory of Department of Microbiology, Faculty of Medicine, University of Indonesia, Indonesia. A total of 35 *Ae. aegypti* female mosquitoes were divided into three pools and blended using dH<sub>2</sub>O solution. RNA samples and RNA DENV (dengue virus) were extracted using QIAamp Viral RNA Mini Kit from Qiagen [13]. Extraction was done at BSC II at BSL following the instructions from the kit. After the extraction, the RNA was aliquoted to 3 20 µl tubes and storage at -80 °C. This study used one step reverse transcription real time PCR. The PCR reagent used in this study was NexQ 7000 from Bioneer. The machine used for RT PCR was Biorad iQ5. The PCR cycle of this reaction was reverse transcription 55 °C for 30 min in one cycle, initial denaturation 95°C for 10 min in one cycle, denaturation 95°C for 30 s and annealing 56°C to 62°C for 1 min in 40 cycles. The RT PCR reaction used SYBR Green I as fluorescent binding dye reporter. Positive control that was used for JEV and CHIKV primer is DNA synthetic as universal control.

## 3. Results

### 3.1 Vector indices

In this study a total of 100 houses were inspected (10 houses/unit) in Sujung village. Immature stage surveys found larvae and/or pupae of *Ae. aegypti*. In 01 neighborhood unit was found 50.0% HI. It was indicated that HI of 01 neighborhood unit was higher than others. Furthermore, in 01 unit positive houses, more immature stages were found than in the others. Of 100 houses, 28% (28/100) positive houses were found as indicated by the HI of 28% (Table 1).

A total of 135 containers were inspected in the same 10 neighborhood units. The maximum of containers was 19 containers found in the 09 units, and the minimum was 10 containers found in 05 and 06A units. The results of the survey showed that in at least one container were found the immature stages in 08 (5.9%, 1/17) and 09 (5.3%, 1/19) units. In the 01 neighborhood unit positive containers (41.7%, 5/12) were higher CI found than that of others. Of 135 containers, 28% (28/100) positive containers were found showed 20.7% (28/135) CI (Table 2).

**Table 1:** House Index of *Ae. aegypti* in Sujung village

Neighborhood Units	Number of Houses	House	
		Positive (+)	Negative (-)
1	10	50.0% (5/10)	50.0% (5/10)
2	10	40.0% (4/10)	60.0% (6/10)
3	10	40.0% (4/10)	60.0% (6/10)
4	10	30.0% (3/10)	70.0% (7/10)
5	10	20.0% (2/10)	80.0% (8/10)
06A	10	30.0% (3/10)	70.0% (7/10)
06B	10	20.0% (2/10)	80.0% (8/10)
7	10	30.0% (3/10)	70.0% (7/10)
8	10	10.0% (1/10)	90.0% (9/10)
9	10	10.0% (1/10)	90.0% (9/10)
Total	100	28.0% (28/100)	72.0% (72/100)

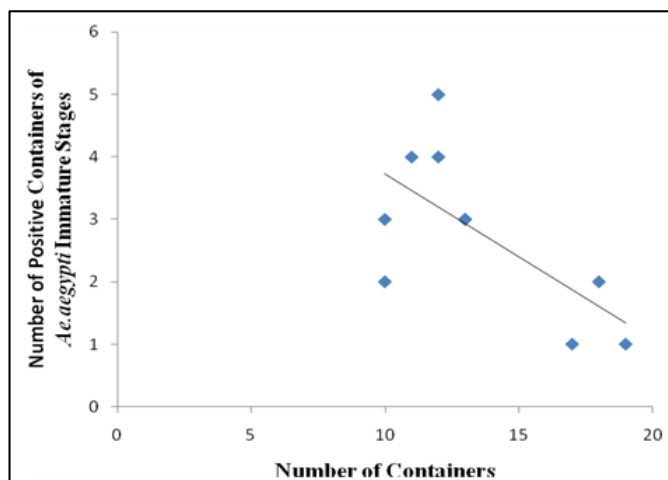
**Table 2:** Container Index of *Ae. aegypti* in Sujung village

Neighborhood Units	Number of container	Container	
		Positive (+)	Negative (-)
1	12	41.7% (5/12)	58.3% (7/12)
2	11	36.4% (4/11)	63.6% (7/11)
3	12	33.3% (4/12)	66.7% (8/12)
4	13	23.1% (3/13)	76.9% (10/13)
5	10	20.0% (2/10)	80.0% (8/10)
06A	10	30.0% (3/10)	70.0% (7/10)
06B	18	11.1% (2/18)	88.9% (16/19)
7	13	23.1% (3/13)	76.9% (10/13)
8	17	5.9% (1/17)	94.1% (16/17)
9	19	5.3% (1/19)	94.7% (18/19)
Total	135	20.7% (28/135)	79.3% (107/135)

Table 3 shows the immature stages of *Ae. aegypti*, consisting of larval and pupal stages, larval stages, and pupal stages. Of 28 positive containers, 64.3% (18/28) containers were found larval and pupal stage, 32.1% (9/18) larvae and 3.6% (1/28) pupae. No significant differences were found immature stage containers in 10 unit of Sujung village ( $p > 0.05$ ).

Figure 1 shows the correlation between number of containers, the independent variable, and number of positive containers of *Ae. aegypti* immature stages, the dependent variable. This study revealed that the number of containers increased and immature stages decreased. Therefore, the trend line showed a

decreasing tendency. This correlation leads to negative Pearson's correlation ( $r = -0.65$ ,  $p$  value = 0.000) because the number of containers, an independent variable, has a tendency to decrease. Therefore, there was a negative significant strong correlations between the number of containers and number of positive containers of *Ae. aegypti* immature stages ( $p < 0.05$ ).



**Fig 1:** Correlation between number of containers and number of positive containers of *Ae. aegypti* immature stages.

**Table 3:** The larval and/or pupal stages of *Ae. aegypti* found in 28 positive containers in Sujung Village.

Neighborhood units	Positive Container	Immature stage-positive containers			One Way Anova p value
		Larvae and Pupae (+)	Larvae (+)	Pupae (+)	
1	5	5	0	0	0.058
2	4	4	0	0	
3	4	4	0	0	
4	3	3	0	0	
5	2	3	0	0	
06A	3	0	3	0	
06B	2	0	2	0	
7	3	0	3	0	
8	1	0	1	0	
9	1	0	0	1	
Total	28	64.3% (18/28)	32.1% (9/28)	3.6% (1/28)	

**3.2 Ovitrap and dengue detection**

A non-attractant ovitrap survey was conducted only once. After 5-7 days, filter papers in each ovitrap were tested for positive or negative *Ae. aegypti* eggs on the paper. A total of 200 non-attractant ovitraps (20 ovitraps/unit) were placed indoors or outdoors of households in Sujung village. Positive non-attractant ovitraps with *Ae. aegypti* eggs found on the paper, were found in 02 (10.0%, 2/20), 05 (5.0%, 1/20) and 07 (10.0%, 2/20) neighborhood units. Of 200 non-attractant ovitraps, 2.5% (5/200) positive ovitraps were found (Table 4). In addition, a total of 30 mosquitos were tested in three pools. The results of detection of dengue virus showed that no dengue virus was found in female *Ae. aegypti* mosquitos (Table 5).

**Table 4:** The results of ovitraps in Sujung village.

Neighborhood Units	Number of Ovitrap	Ovitrap	
		Positive (+)	Negative (-)
1	20	0.0% (0/20)	100.0% (20/20)
2	20	10.0% (2/20)	90.0% (18/20)
3	20	0.0% (0/20)	100.0% (20/20)
4	20	0.0% (0/20)	100.0% (20/20)
5	20	5.0% (1/20)	95.0% (19/20)
06A	20	0.0% (0/20)	100.0% (20/20)
06B	20	0.0% (0/20)	100.0% (20/20)
7	20	10.0% (2/20)	90.0% (18/20)
8	20	0.0% (0/20)	100.0% (20/20)
9	20	0.0% (0/20)	100.0% (20/20)
Total	200	2.5% (5/200)	97.5% (195/200)

**Table 5:** The results of detection of dengue virus in female *Ae. aegypti*.

Pool	Number of female <i>Ae. aegypti</i>	Detection of Dengue Virus	
		Positive (+)	Negative (-)
I	10	0	10
II	10	0	10
III	10	0	10
Total	30	0	30

#### 4. Discussion

DHF remains public health problems and many people of the world live in areas at risk of dengue transmission. DHF cases are increase year by year creating to combat dengue vectors. One of vector control program is to conduct the vector surveillance based on entomological indicators. WHO revealed that vector surveillance is used to evaluate and develop vector control program. The vector surveillance can also obtain data on the geographical distribution and density of the vector in the area [9].

The present study reported that the evaluation vector surveillance is conducted in area of Sujung village where the vector control is rarely conducted by local government and community. In Table 1 showed that all of inspected units of the Sujung village are high density of *Ae. aegypti* larvae because of high HI, CI and BI. A study conducted by Siregar and Makmur [14] in Indonesia reported that entomological surveys in 2011 in two districts North Sumatera, Medan, a district with high DHF incidence and Langkat, a district with low DHF incidence. In Medan HI, CI, and BI were 35.0%, 13.0%, and 43, whereas in Langkat, they were 22.0%, 8.0%, and 30. Similarly, in Sujung village is very low DHF incidence, only one DHF case in 2015 but in Sujung village showed that HI, CI, and BI were 28%, 20.7%, and 28.

Although in Sujung village there is very low risk of dengue transmission, the vector indices such as HI, CI, and BI were above a critical level based on the criteria of Pan American Health Organization [15] which gives three levels of risk for dengue transmission namely low (HI < 0.1%), medium (HI = 0.1%–5%), and high (HI > 5%). Therefore, Sujung village is an area at risk of dengue transmission. In this study was related with other studies of dengue vector surveillances in Indonesia such as in Yogyakarta and Bali [16, 17]. Sulistyawati *et al* [16] reported two urban-villages, Mantrijeron (Intervention site) and Demangan (Control site), in Yogyakarta showed that the vector indices were high. Before interventions, in Mantrijeron were found 19.86% HI and 25.17% HI in Demangan, while after interventions were found 25.53% HI in Mantrijeron and 18.88% in Demangan.

Several studies have found a correlation between entomological indices and dengue transmission in Indonesia [14], Vietnam [18], and India [19]. Bowman *et al* [20] reported that there was little evidence of quantifiable associations between vector indices and dengue transmission that could reliably be used for outbreak prediction, whereas another study found no correlation between entomological indices and dengue transmission [21]. In the present study, since no dengue virus was detected in female *Ae. aegypti* that emerged from larval surveys, the HI >5% found in Sujung village may not correlate with dengue virus transmission.

In this study, we found a correlation between number of containers and number of positive containers of *Ae. aegypti* immature stages. The increased number of containers were found less immature stages so that the trend line has a tendency to decrease. This correlation lead negative Pearson's correlation ( $r = -0.65$ ,  $p$  value = 0.000) because the number of containers, an independent variable, has a tendency to decrease. Therefore, there was a negative significant strong correlation between the number of containers and number of positive containers of *Ae. aegypti* immature stages ( $p < 0.05$ ). It was indicated that female *Ae. aegypti* randomly laid eggs in containers.

In the study, especially in Table 3, was found 28 immature

stage-positive containers; larvae-pupae positive containers (64.3%, 18/28), larvae-positive containers (32.1%, 9/28), and pupae-positive containers (3.6%, 1/28). There was no significant difference of immature stage-positive containers in 10 neighborhood units ( $p > 0.05$ ), because the larval stages rapidly develop into pupal stages in the containers and the containers were man-made breeding habitats containing clean water which were suitable habitat for *Ae. aegypti*. Therefore, the inspections of the containers in this village were found positive containers for larval and/or pupal stages. Furthermore, based on the life cycle of *Ae. aegypti*, the pupal stage may develop into adult stage in two days, so that in this studied areas, 10 units in Sujung village, was found many adult mosquitoes of *Ae. aegypti*.

In this study can not be recorded different types of container such as tank, bucket, jars, bottles, tires, and others were the breeding places for *Ae. aegypti*. A study on vector surveillance conducted by Ferdausi *et al.* [20] in Bangladesh reported that earthen jars, tanks, drums and tires were the mainly containers for larval *Ae. aegypti* breeding. Another study conducted in Uruguay reported that containers can be produced mosquitoes of *Ae. Aegypti* [21]. Therefore, in the water holding container are found not only immature stages but also adult *Ae. aegypti* stages.

In the present study used ovitraps in the dengue vector surveillance. The aim of the use of ovitrap was to know the present of gravid *Ae. aegypti* and *Ae. albopictus* in households of Sujung village. Similarly, the dengue vector surveillance was also used ovitrap in high dengue-risk areas in Taiwan [22]. A study carried out in Brazil by Serpa *et al.* [23] exhibited 24.81% (255/1028) positive ovitrap for the presences of *Aedes spp* eggs. In this study exhibited lower ovitraps with *Aedes spp* eggs (2.5%, 5/200) compared in Brazil.

The ovitrap is a tool of vector surveillances [24]. It is an inexpensive, easily used, and not invasive tool. Application of ovitrap in the field survey should not be employed for more than a week because they could become larval places and may begin producing adult mosquitoes; however, some ovitraps are specifically designed not to produce mosquitoes [25]. Velo *et al.* [26] revealed that ovitrap can be used to monitor species abundances, evaluate a risk of dengue transmission, and manage vector control activities.

#### 5. Conclusions

In conclusion, we found that in Sujung village is high density area of *Ae. aegypti* larvae. The vector surveillance indicated by HI and BI were above a critical value of risk dengue transmission. Results of ovitraps showed gravid *Ae. aegypti* mosquitoes were present in certain households. Although gravid *Ae. aegypti* abundances were present in this village, no dengue virus was detected in this female mosquito. Our study showed that in Sujung village was an area at risk of dengue transmission and should provide insights for vector control programs to be implemented in Sujung village to reduce *Ae. aegypti* population.

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