

RESEARCH ARTICLE

Arbovirus Detection of Adult Female *Aedes aegypti* for Dengue Surveillance: a Cohort Study in Bandung City, Indonesia

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Abstract

Dengue surveillance is an important activity to prevent dengue outbreaks. This activity becomes a significant challenge for the region with limited logistic capabilities. Developing a simple mathematical model to predict the possibility of dengue incidence provides a reliable early warning system. This study compared the correlation between vector (adult female *Aedes aegypti*) and arbovirus detection on a vector to dengue incidence, which generalized linear mixed models tested. The incidence of adult female *Aedes aegypti* and dengue fever cases were interpolated through third-power inverse distance weighting (IDW). A spatial correlation between female *Aedes aegypti* incidence and dengue incidence was obtained from polynomial regression. Collection sites were 16 villages in Bandung city, one of the significant dengue endemic areas in January–December 2017. A total of 8,402 mosquitoes of *Aedes aegypti*, *Aedes albopictus*, and *Culex* sp., with 17% belonging to *Aedes aegypti* as the subject of the dengue virus (DENV) infection test. Data analysis only showed a weak correlation between the numbers of adult female *Aedes aegypti* and dengue incidence. On the other hand, there is no correlation between positive dengue infection of vector and dengue incidence. This study highlights the importance of constant arbovirus surveillance and integrated surveillance methods on all possible dengue vectors to develop an early warning system for dengue incidence.

Keywords: *Aedes aegypti*, arbovirus detection, dengue, Indonesia, surveillance

Introduction

Over the tropical and subtropical regions, *Aedes aegypti* mosquitoes are widely distributed as the vector of arboviruses such as dengue, chikungunya, Zika, and West Nile virus.¹⁻³ *Aedes aegypti* is endemic throughout the tropical and subtropical regions. However, the insect started invading new geographic locations such as Europe for the last 50 years.^{4,5} The transmittance of the dengue virus through *Aedes aegypti* caused approximately 390 million infections a year in 128 countries,⁶ especially Asia,⁷ and caused a significant economic and social burden.⁸ In Indonesia, dengue has been endemic since the first case in 1968, and it has been spreading to all regions in Indonesia, including West Java.⁹ Among Indonesian cities, dengue incidences in Bandung city of West Java are considered higher than in other cities. The total number of reported dengue cases in Bandung city ranged from 3,000 to 6,000 cases annually between 2007 and 2016.¹⁰ The proportion of confirmed serology

from febrile episodes ranged from 7.6 to 41.8% annually, while the average incidence rate of dengue lies was 17.3 cases/1,000 per person (43 times higher than the national average).¹¹

Dengue cases could be reduced by improving detection and prediction through active surveillance.¹² However, instead of active surveillance, passive surveillance has been a widely accepted standard for dengue surveillance in many countries. Since passive surveillance highly depends on the individual seeking clinical treatment, under-reporting dengue cases is highly common for regions with limited medical facilities.⁴ Thus, to overcome monitoring and evaluation problems, developing surveillance methods with predictive power on epidemic risk based on entomological information is needed.¹³

The dengue virus is transmitted through the bites of several vector mosquitoes infected. It transmits the virus through the ovarium, transovarial transmission, or the salivary gland.¹⁴ The virus is infectious and transmissible to humans bitten by mosquitoes in adulthood.¹⁵

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arbovirus surveillance is one of the most vital components in dengue mitigation.¹⁶ This method has been applied for the early detection of potential outbreaks and to identify new arbovirus agents.^{17,18} Detecting the circulation of the dengue virus in mosquitoes could prevent future outbursts of dengue disease, especially in regions with the co-circulation of multiple arboviruses.^{19,20}

Initially, arbovirus detection in mosquitoes was based on the viral antigen, using enzyme-linked immunoabsorbent assay (ELISA) or immunofluorescence assay (IFA). However, it takes time to analyze the samples from remote locations immediately and logistical and cost issues. Molecular-based assays to detect viral RNA in mosquitoes have been preferable as they allow the detection of viruses even after seven days under tropical conditions.¹⁶ Therefore, our study prefers arboviral RNA detection for arbovirus surveillance in mosquitoes.

This paper aims to provide background information on the distribution and impact of *Aedes aegypti* mosquitoes as vectors for arboviruses, particularly dengue, in tropical and subtropical regions. It emphasizes the global spread of *Aedes aegypti* and its invasion into geographic locations. In this study, we compared the spatial incidence of female *Aedes aegypti* and dengue fever cases from 16 villages in Bandung city, Indonesia, to find the correlation between them. The collected female *Aedes aegypti* mosquitoes from those 16 villages were further analyzed for the presence of arboviral RNA to test the hypothesis on the correlation of arbovirus infection on *Aedes aegypti* with the number of dengue cases in the villages. We hope the result of this study could be applied as an alternative approach for mitigating dengue cases.

Methods

This study was conducted in Bandung city, West Java, Indonesia (107°36' east longitude and 6°55' south latitude) with a total area of 16,729.65 ha, 791 m above sea level (ASL). The highest and lowest points are 1,050 m ASL and 675 m ASL, respectively. Bandung has an average annual temperature of 26.8°C and 2,120 mm annual rainfall.²¹ The area is surrounded by mountains that border the region. The city comprises 30 sub-districts and 151 villages, with approximately 2.4 million inhabitants.²² For this study, sampling areas were selected by the stratified random sampling approach.²³

Sixteen villages out of 151 villages in Bandung city were stratified and selected based on altitude, area, population, and the number of dengue cases (Table 1).

We collected mosquitoes from 16 villages across Bandung city using a modified light-type trap and a mosquito attractant solution. Mosquitoes were collected daily for one year and were identified at the Parasitology Laboratory of the Universitas Padjadjaran. Separated adult female *Aedes aegypti* was preserved in RNA later solution at the Parasitology Laboratory of the Universitas Padjadjaran.

We collected dengue hemorrhagic fever cases from primary healthcare posts at the village level (called *puskesmas*) from January to December 2017 from the Bandung City Health Office. The information in the report was based on WHO standard criteria, including age, sex, address, and diagnosis, which were used in this study. The data is contained in the program holder institution.

After the female *Aedes aegypti* mosquitoes were submerged in RNA later solution, ten mosquitoes from each village were pooled into one 1.5 ml microtube. In each microtube, 800 µl of PBS (Takara Bio Phosphate buffered saline (PBS) tablets, Cat# T900) were added. Mosquitoes were then ground and homogenized.

Viral RNA was extracted from each mosquito pool using the Magmax Viral/Pathogen Nucleic Acid Isolation Kit, Cat. No. A48310 and the KingFisher Flex Purification System, KingFisher with 96 Deep-well Head, Cat. No. 5400630. The purified RNA was used for further analysis through multiplex quantitative RT-PCR (qRT-PCR).

Multiplex qRT-PCR was applied to target the RNA of three arboviruses: Zika, dengue, and chikungunya. The qRT-PCR amplification of the targeted viral RNA sequence was done in a 15 µl reaction consisting of 10 µl of Oasig OneStep MasterMix, 1 µl of Multiplex primer/probe mix, and 4 µl of RNase/DNase free water. Samples were incubated in the thermocycler at the following conditions: 55°C for 10 minutes for the reverse transcription process, 95°C for 2 minutes for the enzyme activation process, then repeated for 50 cycles at 95°C for 10 secs and 60°C for 60 secs for denaturation and data collection, respectively.

The incidence of adult female *Aedes aegypti* and dengue fever cases were interpolated through third-power inverse distance weighting (IDW). A spatial correlation between female *Aedes aegypti*

Table 1 Sampling Villages

Villages	Altitude (ASL)	Area (km ²)	Population	Dengue Case
Antapani Wetan	690	115	16,966	47
Ciateul	686	45	10,810	18
Cibaduyut Wetan	689	97.35	6,145	14
Cigadung	750	264.4	26,176	75
Cigondewah Kaler	709	140	22,781	11
Cijawura	670	119.7	22,291	80
Cikutra	706	139.34	22,584	34
Cipedes	891	51	29,415	15
Lebak Siliwangi	792	100	4,238	11
Nyengseret	695	38	11,001	9
Pasteur	790	119	19,132	13
Suka Asih	694	92	21,100	5
Sukabungah	801	51	23,067	15
Sukamaju	730	41.5	10,208	19
Sukapada	720	103	19,526	17
Tamansari	751	102	26,302	26

incidence and dengue incidence was obtained from polynomial regression.

Results

We collected 8,402 mosquitoes of *Aedes aegypti*, *Aedes albopictus*, and *Culex* sp., with about 17% female *Aedes aegypti*, the main subject of this study. The most abundant female *Aedes aegypti* was collected from Nyengseret village (6.9305° S, 107.6016° E), although the highest incidence occurred in Pasteur village (6.8913° S, 107.5993°

E). Both are located near the center of Bandung city, an urban area—the lowest abundant female *Aedes aegypti*, 58, was recorded in Cigondewah Kaler village (Table 2).

We analyzed the feasibility interpolation method and found that IDW is suitable instead of the geostatistical approach of Kriging. This is important since the sampling data does not fit any of the semivariogram models of Kriging, which is circular, spherical, exponential, Gaussian, or linear. Moreover, we found that the mapped variable (female *Aedes aegypti* and dengue fever

Table 2 Mosquito Collection from 16 Villages in Bandung City

No.	Region	<i>Aedes</i> Female	Total Mosquito	% of Total
1	Antapani Wetan	89	614	14.50
2	Ciateul	102	591	17.26
3	Cibaduyut Wetan	71	413	17.19
4	Cigadung	56	212	26.42
5	Cigondewah Kaler	58	1,645	3.53
6	Cijawura	68	1,043	6.52
7	Cikutra	90	285	31.58
8	Cipedes	104	481	21.62
9	Lebak Siliwangi	64	305	20.98
10	Nyengseret	156	595	26.22
11	Pasteur	95	249	38.15
12	Suka Asih	113	366	30.87
13	Sukabungah	83	245	33.83
14	Sukamaju	132	511	25.83
15	Sukapada	104	541	19.22
16	Tamansari	68	306	22.22
	Total	1,453	8,402	17.29

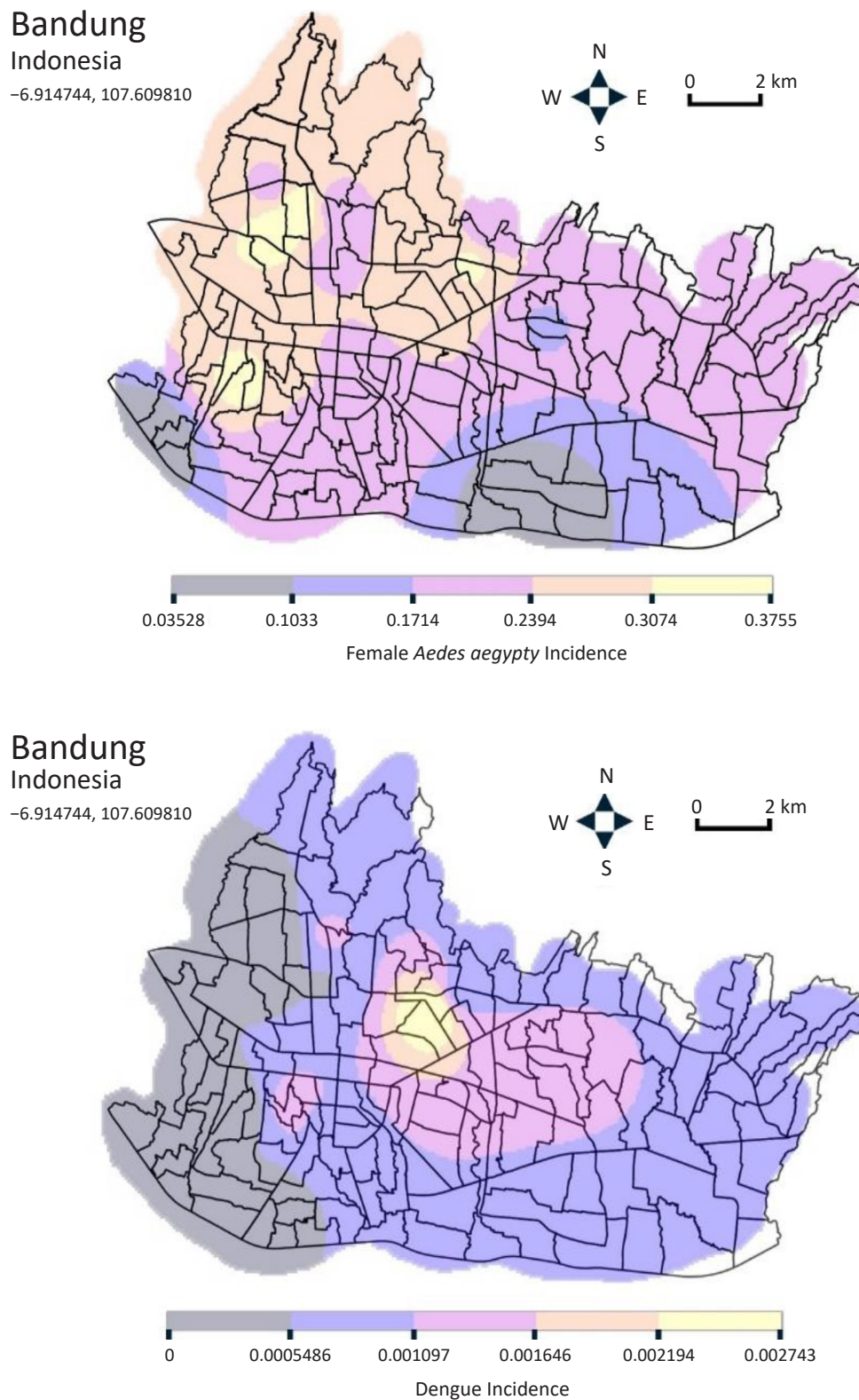


Figure 1 Top: the Interpolated Incidence of Female *Aedes aegypti*,
Bottom: Dengue Fever Incidence
Note: Brighter areas represent higher incidence

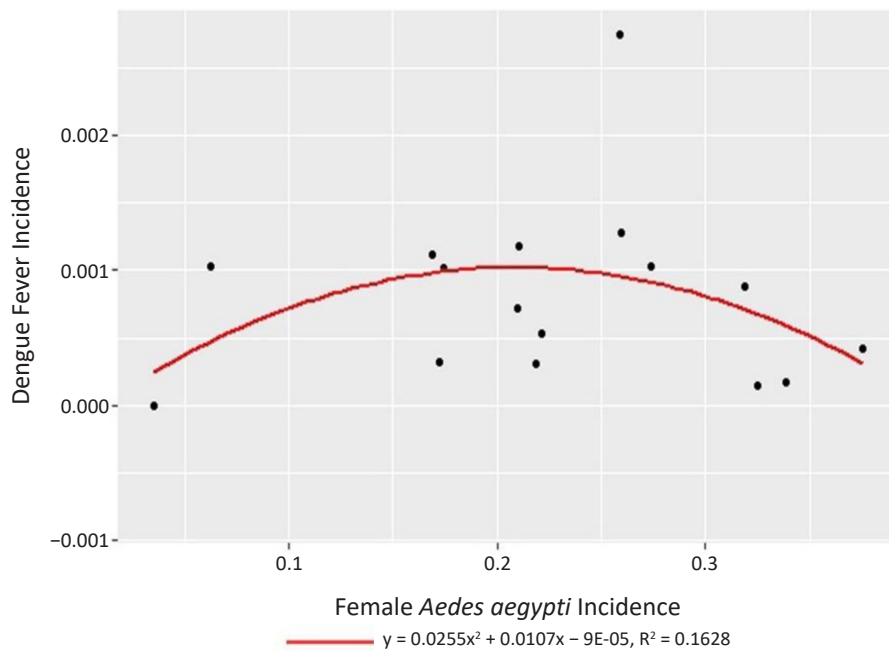


Figure 2 Polynomial Regression between Female *Aedes aegypti* and Dengue Fever Incidence Arbovirus Surveillance

incidence) has an inherent assumption that they will decrease in influence with distance from its initial sampling location. Another parameter defined is the power on which IDW relies. The power of 3 (three) used in this study emphasizes the nearest points. However, the determination

of the power threshold is mainly attributed to when it gives the lowest mean absolute error.

The mapped interpolation in Figure 1 showed higher dengue-infected female *Aedes aegypti* numbers in the northwest of Bandung, while they were lower in the southeastern part. However,

Table 3 Sampling Villages

Villages	Population	Dengue Case	Incidence Rate (%)	Arbovirus Case		
				DENV ^a	ZIKV ^b	CHIKV ^c
Antapani Wetan	16,966	47	0.28	-	-	-
Ciateul	10,810	18	0.17	-	-	-
Cibaduyut Wetan	6,145	14	0.23	-	-	-
Cigadung	26,176	75	0.29	-	-	-
Cigondewah Kaler	22,781	11	0.05	-	-	-
Cijawura	22,291	80	0.37	-	-	-
Cikutra	22,584	34	0.15	-	-	-
Cipedes	29,415	15	0.05	-	-	-
Lebak Siliwangi	4,238	11	0.26	-	-	+
Nyengseret	11,001	9	0.08	-	-	-
Pasteur	19,132	13	0.07	+	+	+
Suka Asih	21,100	5	0.02	-	-	-
Sukabungah	23,067	15	0.07	-	-	-
Sukamaju	10,208	19	0.19	-	-	-
Sukapada	19,526	17	0.09	-	-	-
Tamansari	26,302	26	0.10	-	-	-

Note: ^aDENV: dengue virus, ^bZIKV: Zika virus, ^cCHIKV: chikungunya virus

the interpolation mapping showed contradictory results to dengue case mapping as dengue cases concentrated in the central region of Bandung city. On the other hand, the prediction of different areas is consistent with the hypothesized correlation.

Further correlation analysis showed curvilinear dependence between several female *Aedes aegypti* and dengue fever. A maximum threshold of mosquito incidence in the region affects dengue fever incidence (Figure 2).

We pooled ten mosquitoes per village out of 17% female *Aedes aegypti* of 8,402 mosquitoes collected (1,453 mosquitoes). It was found that arbovirus could be detected in viral RNA extracted from 2 pools of mosquitoes originating from 2 villages (Table 3). The mosquito pool collected from Pasteur village tested positive for Zika, dengue, and chikungunya viruses, while the mosquito sampled from Lebak Siliwangi village tested positive for the chikungunya virus. However, no arbovirus RNA was detected from the mosquitoes obtained from the remaining 14 villages.

Discussion

This report is on the dependence analysis between female and adult *Aedes aegypti* mosquitoes and the incidence of dengue fever in a large urban setting, to our knowledge. Other research reports mainly on the nature of variation from locally acquired dengue cases,^{24,25} dependence from other forms of variables, such as socio-economic factors,²⁶ and the timing of case emergence.²⁷

In this study, the weak spatial correlation between female *Aedes aegypti* and dengue fever cases implies that other governing factors may significantly impact the number of cases that emerged in Bandung. From previous studies and other viewpoints, human settlements are among the most critical factors affecting dengue cases.²⁸ Reasonably, if there is some correlation between human settlements and mosquito incidence in the specific region, there should be notable dependence between variables tested in this study. The proximity of the relationship between humans and mosquitoes is a well-known matter, supported by the notion that blood-requiring insects evolved to prefer the most available and stable source, humans.²⁹ As a result, the approach taken on the factor inference should be more comprehensive than human settlements,

which are environmental aspects, but also other factors from the mobile elements of the system. These include human mobility³⁰ and connectivity between two regions of these studies where the highest incidence of female *Aedes aegypti* and dengue fever cases occurred.

Non-linear dependence from the variables of these studies suggests that mechanisms are yet to be explored. The result was not intuitively obvious, showing that further continuation of mosquito incidence increase in a specific region does not necessarily mean an increase in dengue fever cases. However, the dengue fever case incidence reaches the maximum value at a certain point. There are some reasons to explain this kind of result; one of them is little evidence of quantifiable associations between vector factors and dengue transmission. On the other hand, this can be compared to previous studies on malaria, another mosquito-borne disease with similar onset characteristics. A phenomenon is exhibited in southern Tanzania, where the risk of human infection increased with the entomological inoculation rate (EIR) and vector indices when the parasite prevalence was low. But, when the parasite prevalence was high, an increase in EIR did not appear to increase human infection.³¹ Lastly, another variable that needs to be considered is the herd immunity of the sample. Regardless of the magnitude of entomological indices, the probability of disease transmission will be low if herd immunity is high. Contrarily, if herd immunity is low, any measure of vector incidence will result in an epidemic.

Previous studies have shown that early detection of potential outbreaks of arbovirus through mosquito surveillance is an effective method for dengue mitigation.¹⁷⁻²⁰ In this study, we highlight the possibility of mosquito surveillance for arbovirus as an approach for dengue mitigation in Bandung city, West Java, Indonesia. Results showed that arboviral RNA can be detected in mosquitoes captured from two villages, one of which tested positive for dengue. Interestingly, our findings found no correlation between the positive finding and the number of dengue cases in a village. The mosquito pools collected from the villages with the most dengue cases tested negative for arbovirus. In contrast, the mosquitoes that tested positive for arbovirus infection were collected from the village with a relatively lower number of reported dengue cases.

The positivity rate of mosquitoes infected with

the dengue virus is not the sole factor affecting the number of dengue infections in one region. Higher density of *Aedes aegypti* was not associated with higher dengue infection risk,²² and transmission of the dengue virus can still occur efficiently when the population of *Aedes aegypti* is low.²³ One of the factors affecting dengue virus transmission is the mosquito mortality rate, which changes between seasons. Dengue transmission depends on the probability of whether the mosquito infected with dengue lives long enough for the virus to incubate and further transmit it to susceptible hosts.^{24,25} Environmental conditions and prevention measures are taken to minimize the mosquito population and decrease the livelihood of the mosquitoes, thus affecting the dengue virus transmission rate. The population demographic of each village may also determine their dengue infection rate, as factors such as the age and sex of the hosts affect their susceptibility to dengue infection.²⁶ The trend for dengue infection in Indonesia in the last 22 years showed that the adult population aged 15 years and above is more prone to dengue infection than children, which shifted from the previous trend, where children were more susceptible.²⁷ Therefore, even if the number of infected mosquitoes in a region increases, the number of dengue incidences will not necessarily increase, depending on the host's susceptibility. Another possible explanation of the result is the possibility of *Aedes albopictus*, which is not included in this study, as a vector of dengue. Studies showed the increasing role of *Aedes albopictus* as a dengue vector in various countries,^{28,29} and their population is correlated to the lack of sanitation in Indonesia's high human population density region.³⁰ Although the level of dengue virus infection in *Aedes albopictus* is usually significantly lower than in *Aedes aegypti*, the higher population of this species may improve the chance of dengue virus infection. However, this hypothesis should be assessed by future studies.

Mosquito incidence is also one of the determinants of virus transmission efficiency. A higher biting rate was observed in smaller mosquitoes in contrast to larger ones, thus increasing the probability of infecting hosts. Adult mosquitoes with higher incidence have a lower positivity rate, which was also implied by the results of this study. Consequently, it is less effective for larger female mosquitoes to be targeted in dengue mitigation. Since this study

only considered adult female mosquitoes as the target for the surveillance, further advancement in arbovirus surveillance in mosquitoes to mitigate dengue outbreaks should also consider the potential of mosquito larvae as the target for surveillance and mitigation. Due to the transovarial transmission of the dengue virus in mosquitoes, controlling mosquito larvae could be essential as immature stages have the potential to be a virus reservoir.¹⁵

Due to the complexity of vector-host interaction in dengue transmission, the correlation between vector incidence and positive infection rate could be determined more accurately if mosquito surveillance for arbovirus is performed over multiple periods.²² However, it could be very time consuming and not cost-effective as a routine for dengue surveillance. Arbovirus surveillance in mosquitoes described in this study has shown the possibility of a more cost-efficient and less laborious approach for routine mosquito surveillance for circulating arbovirus in urban areas such as Bandung city.

Conclusion

This study highlights the importance of constant arbovirus surveillance and integrated surveillance methods on all possible dengue vectors to develop an early warning system for dengue incidence.

Conflict of Interest

No conflicts of interest.

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