Original papers

Altitudinal distribution of *Aedes* indices during dry season in the dengue endemic area of Central Java, Indonesia

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ABSTRACT. *Aedes* mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus*, are the primary and secondary vectors of dengue viruses in Indonesia, with transmission occurring by sucking blood. The density of the vectors is influenced by season and rainfall, but limited by altitude. The aim of the study is to describe the density and distribution of dengue vectors during the dry season based on the altitudes of recent dengue cases in five regencies of Central Java Province, Indonesia. Mosquito larvae and pupae were collected from the indoor and outdoor water containers from 253 houses within 50 m of houses occupied by a dengue patient. A considerable dengue vector population was found in all localities and altitudes based on the *Aedes* indices: an HI of 41.7% (15.0–70.6), CI of 33.6% (8.1–69.6) and BI of 57.1 (15.0–94.1). The highest indices were found in the highest altitude settlement; as the most common larval habitat in this village was a large-sized cement tank, larvivorous fish can act as effective predators in this case. This finding indicates an expansion of the dengue problem from low to high altitudes, causing a high potential for dengue transmission in all of the localities.

Key words: *Aedes aegypti*, dengue vector, dry season, altitudinal distribution

Introduction

Dengue has been reported in all continents of the world, with the majority of cases being found in Asia [1]. The number of new cases has been estimated to be as many as 400 million persons each year, and more than 125 countries within the WHO region are endemic for dengue [2]; these lie mainly in the tropical and sub-tropical region, between latitudes 35°S and 35°N, placing 2.5 billion people at risk [3]. Temporal patterns of dengue occurrence have changed in the last decade: while dengue incidence used to peak at the end of the rainy season, the incidence now disperses throughout the year [4–6]. This phenomenon has been seen in many countries, and has placed an increasing burden on public health [1].

The incidence of dengue in Indonesia has fluctuated over the last eight years. The Ministry of Health of Indonesia stated that dengue incidence per 100,000 people increased from 50.75 in 2015 to 77.96 in 2016, while the targeted value is 49 per 100,000 people [7,8]. The area affected by dengue rose from 86.77% of districts or municipalities all over Indonesia in 2015 to 90.08% in 2016 [8]. Dengue cases have dispersed from epicenters in urban areas to peri-urban and rural areas [9]; for example, the range has dispersed from Bangkok to the surrounding localities in Thailand at an estimated velocity of 148 kilometers per month [13].

The occurrence of dengue has also spread from low altitudes to more than 1,000 m above sea level [4,10], under the influence of increases in minimum temperature, and population migrations resulting from changes in transportation, globalization,

trading, social-economic condition, housing and viral evolution [2]. An average monthly minimum temperature of more than 14.8°C is a very conducive physical factor for dengue occurrence, and dengue endemicity is influenced by rainfall intensity [11]. Its incidence also affected by humidity, duration of daylight and wind velocity [12]. These physical factors can be the good predictors for dengue vector dynamics [13].

Dengue virus is transmitted by sucking-blood activity of *Aedes* mosquitoes, with *Aedes aegypti* and *Aedes albopictus* being the main primary and secondary vectors in Indonesia [7,14]. The presence of larvae and pupae of *Aedes* mosquitoes can be predicted by water storage use, rainfall intensity and minimum temperature [13]. Their population density is influenced by the season, with *Aedes* indices being higher in the post-monsoon than pre-monsoon season [15]. However, decreasing the *Aedes* population density in the dry season could result in more effective transmission of dengue virus [16]. The *Aedes* mosquito is very adaptive to environmental change, especially those associated with climate, settlement quality and socio-cultural change, and they are able to breed throughout the year [3,17–19] in both indoor and outdoor water containers [20]. Cross-country studies in Latin America have found that the infrequent use of a large-size, uncovered water tank outside the home, which is filled with water during the rainy season, has the effect of preserving productive breeding sites, particularly during the dry season [21].

Vector control is the most effective method to prevent dengue infection. Its aim is to reduce the population density of *Aedes* as indicated by the House Index (HI) < 5% [7]. Some studies have found high *Aedes* indices in many countries. One study of six countries in South and Southeast Asia found *Aedes* indices to be higher in the wet season than dry season [22]; however, a two-year study in Ubonratchathani, Thailand found the opposite: HI, CI and BI were higher in the dry season than the wet season [23].

In Indonesia, climate change has resulted in the spread of dengue vector throughout the year due to changes in rainfall in the dry and wet seasons. A study in six districts of Central Java Province found *Aedes* indices to range from 27.3 to 55.2, 19.1 to 53.8 and 44.8 to 72.7 for HI, CI and BI, with lowest density found at Temanggung district, at an altitude above 500 meters above sea level [24]. The contradictory results of those studies demand a deeper analysis of the dengue vector situation.

Dengue vectors, mainly *Aedes aegypti*, were believed to be rarely found at high altitudes of more than 1,000 m above sea level [14]. However, this species has been found at various altitudes up to 2,130 m ASL, although a high population density was encountered above 1,000 m ASL and a low population density at 2,000 m ASL or above [25–27]. The House Index ranged from 17 to 62 percent at an altitude more than 1,000 m ASL in Mexico, and it was associated with temperature index [25]. A similar phenomenon was also reported from Indonesia, where the dengue vectors *Aedes aegypti* and *Aedes albopictus* were found at the various elevations up to 1,000 m ASL [4,24]. *Aedes aegypti* population density has also been found to correlate positively with altitude [28], although the values of indices were not stated.

The change in the epidemiological profile of dengue in Central Java Province, Indonesia is worth noting: the incidence of dengue occurs throughout the year, the transmission spread from the epicenter to the surrounding areas, and the outbreak occurred at the high altitude areas above 1,000 m ASL. As the *Aedes* mosquito has an important role in the transmission of dengue virus, it is important to analyze the influence of dengue vector density, based on *Aedes* indices, the characteristics of the productive larval sources, and the altitudinal distribution of dengue endemic areas during the dry season. This data provides an insight into recent changes in dengue vector density and its related factors at various elevations, and improving dengue control programs. The aim of the study is to describe the density and distribution of dengue vectors during the dry season based on the altitudes of recent dengue cases in five regencies of Central Java Province, Indonesia.

Materials and Methods

Field of study. This cross-sectional study was conducted in four districts (Kudus, Semarang, Pemalang, and Tegal) and one municipality (Semarang municipality) which have undergone an increasing occurrence of dengue during 2016. Kudus and Tegal District and Semarang municipality are low-altitude regions, while Pemalang and Semarang districts are at various elevations from 15 to 1,500 m ASL. Three endemic villages with different altitudes were selected based on dengue case data in the Public Health Center

Table 1. Types of water container among 253 houses in Central Java Province, Indonesia

No.	Type of container	Larva existence	
		n	%
1	Bathroom water container	196	45.4
2	Toilet water container	2	0.5
3	Drum	5	1.2
4	Earthen jar	56	13.0
5	Bucket	138	31.9
6	Dispenser reservoir	6	1.4
7	Refrigerator reservoir	3	0.7
8	Discharged tin	3	0.7
9	Discharged glass/cup	3	0.7
10	Used tire	4	0.9
11	Used aquarium	1	0.2
12	Large-sized cement tank	10	2.3
13	Flower pot	2	0.5
14	Used bucket	3	0.7
	Total	432	100.0

office in each district or municipality.

Sampling and entomological surveys. Household cluster surveys were carried out in fifteen villages for four months from May to August 2016. Each cluster consisted of the house of a dengue patient and fourteen to twenty-two houses surrounding it within a 50m radius. A total of 253 houses were included in the dengue case-based entomological surveys. After informed consent was obtained from the household, the domestic water storage and other water containers, both indoor and outdoor, were examined in each house. A range of water storage characteristics including type, material, number, wall color and placement were recorded, and the existence of larvae and pupae in the container were recorded. The larvae and pupae were collected in plastic bottles, labeled according to village, and transported to the Epidemiology Laboratory of the Public Health School of Universitas Muhammadiyah Semarang. The larvae and pupae were subjected to morphological identification based on the single larva method. The insects were maintained into the adult stage for later insecticide susceptibility testing (studies in progress).

Data analysis. All of the data obtained was entered into a Microsoft Excel spreadsheet. SPSS for Windows was used for statistical analysis of *Aedes* indices and larva existence and distribution based on the study sites, altitudes, and container characteristics.

Ethical statement. Approval was obtained from the Provincial and Local Health Office for the study to be performed and informed consent was obtained from all households. This study did not involve the collection of specimen data from human participants.
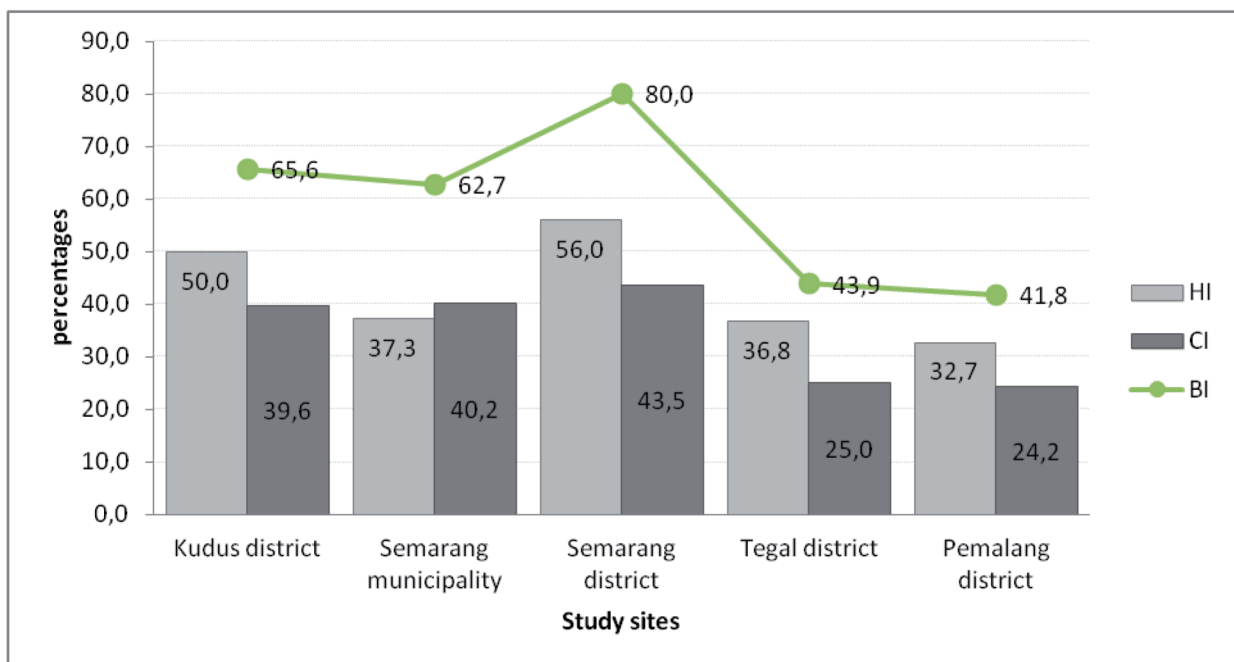

Fig. 1. Distribution of the traditional *Aedes* indices among study site showed that the indices more than the prophylaxis threshold level study sites are at risk of dengue transmission, mainly Semarang district

Table 2. Distribution of larva positive houses based on study sites and altitudes

Variables	Larvae positive house		p
	Yes	No	
Study site	n (%)	n (%)	
Kudus district	16 (50.0)	16 (50.0)	0.093
Semarang municipality	22 (37.3)	37 (62.7)	
Semarang district	28 (56.0)	22 (44.0)	
Tegal district	21 (36.8)	36 (63.2)	
Pemalang district	18 (32.7)	37 (67.3)	
Altitudes (m asl)			
0 – 100	39 (33.6)	77 (66.4)	0.049
101 – 500	40 (45.5)	48 (54.5)	
501 – 1,000	16 (47.1)	18 (52.9)	
> 1,000	10 (66.7)	5 (33.3)	

Results

In total, 253 houses were examined. These houses were found to include 432 water containers: 395 (91.4%) indoors and 37 (8.6%) outdoors. *Aedes aegypti* larvae were found in 105 (41.5%) of the houses and 146 (33.8%) of the water containers. The most common types of water container were bathtub, bucket, earthen jar, large cement tank and dispenser reservoir (Table 1). Data analysis found the traditional *Aedes* indices to range from 32.7 to 56.0%, 24.2 to 43.5%, and 41.8 to 80.0% for HI, CI and BI, respectively. The indices indicate that all study sites are home to large *Aedes* populations, with the lowest and highest of *Aedes* indices being found at Pemalang and Semarang districts, respectively (Fig. 1). These indices tend to increase with altitude, although this trend was no significant (Table 6).

The presence of *Aedes aegypti* larvae in the examined houses did not vary significantly according to study site, but the numbers did change significantly with altitude (Table 2). The highest number of larvae present in the water container was found at the highest altitude (>1,000 m ASL). The presence of *Aedes aegypti* larvae in containers was significantly associated with study site, altitude, type of container and location of container (p<0.05), but not with wall color of container or water source (p>0.05) (Table 3 and Table 4). Nine of the water container types distributed at various altitudes were found to be the productive larval habitats (Table 5). Flower pots and dispenser reservoir are productive larval habitats when found at an altitude of 101–500

Table 3. Distribution of larvae positive container based on study sites and altitudes in Central Java Province, Indonesia

Variables	Larvae existence		p
	Yes	No	
Study site	n (%)	n (%)	
Kudus district	21 (39.6)	32 (60.4)	0.008
Semarang municipality	37 (40.2)	55 (59.8)	
Semarang district	40 (43.5)	52 (56.5)	
Tegal district	25 (25.0)	75 (75.0)	
Pemalang district	23 (24.2)	72 (75.8)	
Altitudes (m asl)			
0 – 100	50 (26.7)	137 (73.3)	0.005
101 – 500	57 (38.0)	93 (62.0)	
501 – 1,000	24 (34.3)	46 (65.7)	
> 1,000	15 (60.0)	10 (40.0)	

Table 4. Characteristics of larvae positive containers

Characteristics of containers	Percentage of larva existence		p
	Yes	No	
	n (%)	n (%)	
Type of water container			
Cement tank	60 (30.2)	139 (69.8)	0.000
Bucket	41 (29.1)	100 (70.9)	
Earthen jar	20 (35.1)	37 (64.9)	
Drum	4 (100.0)	0 (0.0)	
Used tire	4 (100.0)	0 (0.0)	
Flower pot	1 (100.0)	0 (0.0)	
Dispenser reservoir	1 (16.7)	5 (82.3)	
Junk/wreck	8 (100.0)	0 (0.0)	
Large-sized cement tank	7 (70.0)	3 (30.0)	
Refrigerator reservoir	0 (0.0)	0 (100.0)	
Location of water container			
Indoor	115 (29.1)	280 (70.9)	0.000
Outdoor	31 (83.8)	6 (16.2)	
Wall color of water container			
Dark	69 (34.8)	129 (65.2)	0.373
Light	77 (32.9)	157 (67.1)	
Water source			
Rain water	6 (54.5)	5 (45.5)	0.285
Well	77 (32.1)	163 (67.9)	
Tap water	63 (34.8)	118 (65.2)	

m ASL, while a large cement tank (giant container) is an important larval source when found in mountainous areas, like Gombong village in the southern part of the Pemalang district. It was not found such giant container in areas lower than 1,000 m ASL. Predatory fish were found in 30% of the large cement tanks which were negative for larvae.

Discussion

This study implemented an easy vector surveillance method which was applied to evaluate the vector abundance in areas endemic for dengue. The WHO recommends the method for understanding population density, general distribution and larval habitats of the disease vectors, namely *Aedes aegypti* and *Aedes albopictus* [29]. The surveillance method yelded *Aedes* indices used to determine vector abundance and the need for control strategy. A house index – 5% is regarded as the prophylactic threshold of dengue virus transmission by the Ministry of Health of Indonesia

Table 5. Distribution of larvae positive container based on types and altitudes

No.	Types of water container	Larva existence (%) based on altitudes (m asl)			
		0 – 100	101 – 500	501 – 1,000	>1,000
1	Cement tank	34.8	32.4	13.5	50.0
2	Bucket	13.7	40.5	68.4	14.3
3	Earthen jar	31.6	35.7	25.0	100.0
4	Drum	–	100.0	–	100.0
5	Used tire	100.0	–	100.0	–
6	Flower pot	–	100.0	–	–
7	Dispenser reservoir	–	50.0	–	–
8	Junks/wrecks	–	100.0	100.0	100.0
9	Large-sized cement tank	–	–	–	70.0

Table 6. Traditional *Aedes* indices based on altitudes

Aedes Indices	Altitudes (m asl)	Minimum	Maximum	Means	p
HI	0 – 100	15.00	50.00	33.63	0.255
	101 – 500	25.00	70.60	47.70	
	500 – 1,000	41.20	41.20	41.20	
	>1,000	66.70	66.70	66.70	
	Total	15.00	70.60	42.56	
CI	0 – 100	8.10	54.80	27.57	0.472
	102 – 500	17.80	69.60	39.58	
	500 – 1,000	35.70	35.70	35.70	
	>1,000	56.00	56.00	56.00	
	Total	8.10	69.60	35.33	
BI	0 – 100	15.00	85.00	43.22	0.239
	103 – 500	35.70	94.10	66.85	
	500 – 1,000	58.80	58.80	58.80	
	>1,000	93.30	93.30	93.30	
	Total	15.00	94.10	58.04	

[7], who note that dengue virus transmission still occurs at an HI value of 2% [29], while the WHO recommend HI 10% as a high level [30].

Our results provide further local information on the presence and distribution of dengue vectors in Central Java Province, Indonesia. *Aedes* mosquitoes were found to be distributed at all altitudes in the study area up to 1,200 m ASL, indicating the influence of climate change. This is the highest altitude that dengue has yet been identified and the first time such conditions have been recorded in the area; both indicate changes in dengue vector distribution in Indonesia occurring in response to climate change, as also noted in other countries [21,27]. These figures are in line with a Ministry of Health of Indonesia report indicating that the percentage of districts or municipalities with dengue has risen from 84.74% in 2014 to 86.77% in 2015 [7]. Similar trends have been identified in previous studies conducted in several localities in Indonesia [28,31].

Our findings complement previous reports of *Aedes* indices from neighboring areas such as the Banyumas district [31], and are in line with *Aedes* population density in major transportation lines in Central Java Province [24]. Furthermore, our results show higher *Aedes* indices in the districts with a high level of dengue endemicity, although no significant difference was found between different locations and their elevation. Although traditional *Aedes* indices do have some disadvantages, they serve as a straightforward and quick method for assessing and evaluating the prevalence of *Aedes*

mosquitoes and environmental sanitation [30]. However, the resistance status and genetic diversity of *Aedes aegypti* remain poorly understood.

Our findings show that people in mountainous localities use large, open cement tanks to store clean water, which have become the most common larval habitat at high altitudes. A similar phenomenon has been observed in Latin American countries, where the people have similar habits regarding daily use of clean water and the dengue vector abundance is similar [21]. These findings complete those obtained in a previous report of dengue vector distribution and population density in Central Java Province. In addition, it was found that the presence of larvae predatory fish in the water container can be an effective and efficient method for controlling dengue vectors in mountainous areas. This method should be promoted as the best strategy in controlling dengue vector in large-sized container with large amount of water.

The existence of *Aedes* mosquitoes at high altitudes up to 1,200 m ASL indicates that the physical and biological factors in the area are capable of supporting the mosquito life cycle. *Aedes* mosquitoes have not previously been found at altitudes of 1,000 m ASL due to the low temperature, which impedes the *Aedes* life cycle [32]. The development of *A. aegypti* larvae was influenced by temperature, larval density, diet and the interaction among those factors [33]. The optimum temperature and humidity range for this species is 23–27°C and 80%, and temperatures higher than 35°C and humidity less than 60% have

a negative effect on development [34,35]. A study in Brazil showed that temperature affects the embryo, larva, pupa, fecundity and longevity of *A. aegypti* mosquitoes. This species will develop optimally in similar temperature range [36]. Favorable temperatures will accelerate the metabolic process of mosquitoes, resulting in increased bloodsucking rate, fecundity and population density, while the humidity will influence mosquito survival and activity [16].

Climate change has been reported around the world, resulting in warmer temperatures being observed at high altitudes. This phenomenon triggers the wide spread environmental change that is suitable for increasing dengue vector expansion at present and the next decades progressively [37]. Similarly, the further spread of the dengue vector throughout altitudinal areas has been observed in Central Java Province, Indonesia resulting in outbreaks of dengue cases in several localities.

All altitudes up to 1,200 m ASL in the studied area are at high risk of dengue transmission because the vectors were found at high population densities during the dry season, indicating the effects of climate change. The most common and frequent larval habitat within the study sites is bathroom water container (small or normal-sized cement tank), bucket, earthen jar, large-sized cement tank, dispenser reservoir and drum. A large-sized cement tank is the most common larval habitat at the mountainous locality, and these can be controlled effectively by using larvivorous fish. This finding can be used to adapt public health activities for conducting vector control strategies.

Acknowledgements

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