

Original Study Article

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Analysis of entomological indicators and distribution of *Aedes aegypti* larvae in dengue endemic areas

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Abstract

Background. The cause of dengue fever's endemicity is vector larvae density, with *Aedes aegypti* as the prime vector. Bandung municipality is a high dengue fever endemic area. Hence, studying the habitat characteristics of the *Aedes* mosquito is essential to controlling the populations of mosquitos.

Purpose. This study aimed to identify the aedes larvae breeding sites and the relationship between the breeding risk index, hygiene risk index, maya index, and the existence of larvae with the incidence of dengue fever.

Method. The design used in this research was a cross-sectional survey. The sampling technique used simple random sampling. The quantity of the sample was 544 directly observed houses.

Results. The entomological parameters obtained were house index (23.89%), container index (7.81%), Breteau index (50.73%), and larva free number (76.10%). The breeding risk index, hygiene risk index, and maya index are in the low category. The chi-square test conveyed that the breeding risk index, maya index, and the existence of larvae were significantly associated with the incidence of dengue fever.

Conclusion. The entomological index may influence the high incidence of dengue fever. These findings and results may help the authorities to improve mosquito nest eradication in attempts to prevent dengue transmission.

Keywords: *Dengue, Aedes aegypti, Dengue transmission, Entomology Index, Mosquito larvae*

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Оригинальное исследование

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Анализ энтомологических показателей и распространение личинок *Aedes аегурти* в эндемичных по лихорадке денге районах

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Аннотация

Введение. Причиной эндемичности лихорадки денге является плотность популяции личинок комаров-переносчиков, основным из которых являются *Aedes aegypti*. Муниципалитет Бандунг является эндемичным районом с высоким уровнем заболеваемости лихорадкой денге. Следовательно, изучение характеристик среды обитания комаров рода *Aedes* имеет важное значение для контроля популяций комаров.

Целью данного исследования было выявление мест размножения личинок комаров рода *Aedes* и взаимосвязь между индексом риска размножения, индексом гигиенического риска, индексом Майя и наличием личинок с заболеваемостью лихорадкой денге.

Материалы и методы. В данном исследовании использовался метод перекрёстного опроса. Объём простой случайной выборки составил 544 дома.

Результаты. Полученными энтомологическими показателями были индекс дома (23,89%), индекс контейнера (7,81%), индекс Брето (50,73%) и количество объектов, свободных от личинок (76,10%). Индекс риска размножения, индекс гигиенического риска и индекс Майя относятся к низкой категории. Тест χ^2 показал, что индекс риска размножения, индекс Майя и наличие личинок были в значительной степени связаны с заболеваемостью лихорадкой денге.

Заключение. Энтомологический индекс может влиять на высокую заболеваемость лихорадкой денге. Эти выводы и результаты могут помочь властям оптимизировать программу по ликвидации мест размножения комаров с целью предотвратить распространение лихорадки денге.

Ключевые слова: лихорадка денге, *Aedes aegypti*, распространение лихорадки денге, энтомологический индекс, личинки комаров

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Dengue is still a global public health issue, particularly in tropical and subtropical areas, including Indonesia as one of the dengue endemic countries [1]. Dengue is a disease caused by the dengue virus which has the fastest spread worldwide [2]. The global incidence of dengue has risen significantly, with approximately half of the world's population at risk. Although an estimated 100–400 million infections occur yearly, more than 80% are generally milder and asymptomatic¹.

Dengue is a disease which causes death due to bleeding and hemodynamic disorders. The primary vector of dengue is the *Aedes aegypti* mosquito, whereas the potential vector is *Ae. albopictus*. These mosquitoes are also vectors of chikungunya, yellow fever, and zika virus [3]. Specific medicines and vaccines that are efficacious for dengue are still under investigation, since no particular drug is efficacious in the treatment of dengue [4].

Indonesia is a dengue-endemic country. 248,127 cases were reported in 2019. Meanwhile, until July 2020, the number of cases had reached 71,633 cases². An epidemiological study of dengue fever in Bandung Municipality stated that the existence of *Ae. aegypti* mosquito larvae highly influences the high number of infection cases [5]. Prevention and management of den-

gue fever depends on effective vector control measures. Sustained community involvement can substantially improve vector control efforts.

Multiple factors have been implicated in the global resurgence of dengue fever. These include failure to control aedes populations, uncontrolled urbanization, and unprecedented population growth [6]. The seasonal dynamics of *Ae. aegypti* population sizes generally have a positive relationship with climatic variables such as temperature, rainfall, and relative humidity [7]. Previous studies have indicated that the distribution pattern of dengue can be affected by various factors, one of which is the existence of mosquito vectors that can be assessed through entomological indexes such as: House Index (HI), Container Index (CI), Breteau Index (BI), and Mosquito larva Free Rate (ABJ). High dengue disease transmission is encouraged by the high density of *Ae. aegypti* mosquitoes [7, 8].

Current prevention efforts include Mosquito Nest Eradication (Pemberantasan Sarang Nyamuk, or PSN), but implementation still needs to be optimized. One of the efforts that have proven effective is to reduce and suppress the mosquito population. WHO states that effective dengue prevention is achievable by controlling vectors from larvae to adult mosquitoes [9]. In dengue eradication programs, the typical larval survey is the investigation of *Ae. aegypti* larvae in residential and communal areas using the single larva method [10].

With an increasing tendency of dengue incidence, especially in Bandung Municipalities, the research focus has been on case tracing and control of *Ae. aegypti*.

¹ WHO. Dengue and severe dengue; 2023. URL: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>

² Kementerian Kesehatan Republik Indonesia. Kemenkes R.I. Profil Kesehatan Indonesia 2020. Jakarta;2021. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/profil-kesehatan-indonesia/Profil-Kesehatan-Indonesia-Tahun-2020.pdf>

Therefore, there is a need to conduct a study of distribution patterns based on entomological indicators. This program will be very useful when used as basic literature. It can also form the basis for making vector control policies more precisely targeted in an effort to prevent dengue fever. Vector population density can help programs determine the rate of speed of the dengue virus spread in a particular area [11]. This research aims to identify the breeding sites of *Aedes* larvae and the association of the Breeding risk index (BRI), Hygiene risk index (HRI), Maya index (MI), and the occurrence of larvae with the incidence of dengue fever. The findings of this research can serve as a foundation for dengue fever prevention efforts through effective mosquito nest eradication efforts.

Materials and methods

Research design

This research used a cross-sectional design. This study was conducted in Bandung City in March–July 2022. This study surveyed each endemic area. Each stratification selected nine sub-districts based on a purposive sampling design.

Sampling technique

The population in this research was heads of households from all residents of Bandung City. The sample selection of endemic sub-districts was based on sub-districts with the highest number of cases by considering existing incidence rate data and stratification based on the Bandung City health office in 2021. 544 houses were taken using the Sample Size for One Sample Test of Proportion formula (level of significance 5%, power 90%). The sample houses were chosen using a random sampling method from the combined list of residents owned by each sub-district. A house was sampled, if selected as a sample and willing to be visited.

Entomology survey

Surveys were conducted on larvae by examining all water storage containers accessible from respondents' homes, in order to determine the most effective and efficient types of containers for mosquito breeding. Surveys were conducted on wet containers filled with water at the time of the survey. Samples of larvae found were collected using a net (tea strainer) and then placed in small plastic jars filled with water. Each sample jar was labeled with the identification number, date, location, and number of larvae gathered. Breeding sites of the mosquito species collected were recorded in pre-determined survey data sheets during sample collection. Containers were recorded as positive for *Aedes*, if larvae were found in the water storage containers.

Containers classification

Containers were categorized into three groups based on their purpose: Controllable containers, dis-

posable containers, and under-controllable containers. Water storage containers recorded in the survey as controlled containers are places that can be maintained or controlled, such as bathtubs, water tanks, buckets, jerry cans, wells, flowerpots, drums, refrigerators, dispensers, barrels, animal drinkers, crocks and gutters. Used containers are waste or places that are no longer used, such as used cans, tires, bottles, buckets, jars, glasses, and drums. Furthermore, containers such as fishponds and aquariums were deemed under-controllable.

Larvae identification

After being collected, the larvae were taken to the parasitology laboratory of Bhakti Kencana University. The larvae were identified under a microscope in the laboratory. Species identification was finalized using standard identification keys explained in the literature "Pictorial keys for identifying mosquitoes (Diptera: *Culicidae*) associated with Dengue Virus Transmission" [12]. Furthermore, laboratory results were then recorded on the appropriate survey sheet.

Data analysis

Maya index and entomological index analysis were used to illustrate the transmission risk of dengue. Dominant containers were identified by data collection on the containers with the highest number of positive larvae found inside and outside the house. Containers in this regard were grouped into two categories: controlled containers and used containers. The Maya Index in this research was determined using two indicators: BRI and HRI. BRI is each house's proportion of controllable sites. BRI is obtained by dividing the number of controllable areas examined by the average number of containers in the house. The HRI is each house's proportion of disposable sites. HRI is calculated by dividing the number of disposable sites in the examined homes by the average number of containers in the home [13]. BRI, HRI, and MI values were classified into three categories: high, medium, and low, based on Lozano's distribution (2002). MI is determined from the BRI and HRI values in each house arranged in a 3×3 matrix [14].

Entomological indicators, namely HI, CI, and BI, are categorized based on the larval density index according to the density figure (DF) value in the low, medium, and high categories [17]. CI is an index used to define the number of larvae-positive containers out of all containers examined (Number of larvae-positive containers \times 100% / Number of containers discussed). HI is an index used to identify the number of larvae-positive houses out of all homes inspected (Number of larvae-positive homes \times 100% / Number of homes inspected). BI is an index used to determine how many larvae-positive containers out of all the homes examined (Number of larvae-positive containers \times 100% / Number of homes examined). The entomological index defines the risk of infection based on DF. The low

Table 1. Density of *Aedes Aegypti* Larvae Based on DF and Indicators of HI, CI, and BI

DF	HI	CI	BI
1	1–3	1–2	1–4
2	4–7	3–5	5–9
3	8–17	6–9	10–19
4	18–29	10–14	20–34
5	30–37	15–20	35–49
6	38–49	21–27	50–74
7	50–59	28–31	75–99
8	60–76	32–40	100–199
9	–	–	–

Source: Service, MW. Mosquito Ecology Field Sampling Methods. Chapman and Hal.

density is categorized as DF 1, medium density as DF 2–5, and high density as DF 6–9 [11]. The determination of the density of index larvae based on HI, CI, and BI taken from the MW Service can be seen in **Table 1**. MI analysis is determined by identifying controlled and uncontrolled water reservoirs, namely the categories of controllable sites and disposable sites.

The Chi-Square test was used for further analysis. This test was used to analyze the relationship between the Maya index and the occurrence of larvae with the dengue incidence, with a significance level of 5%.

Results

This study was conducted on 544 homes and resulted in an incidence rate of 41% (**Fig. 1**). The elevation of dengue incidence is relevant to the number of larvae found in the houses. A total of 276 containers containing larvae were found. The majority of mosquito larvae were found in bathtubs (52), dispensers (40), refrigerators (30), buckets (24), bottles (23), and used tires (13). At the same time, wells, barrels, crocks, used

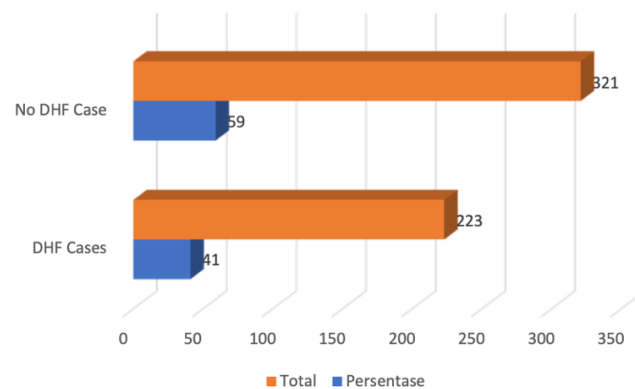


Fig. 1. Prevalence of dengue in Bandung municipality.

jars, used cups, used drums, fishponds, and aquariums were not found with any larvae at all. Most mosquito larvae were found in controllable containers (**Fig. 2**).

The MI status is based on potential mosquito breeding sites (BRI) and environmental cleanliness (HRI). MI analysis (**Table 2**) conveys that BRI, HRI, and MI values di Kota Bandung are primarily in the low category.

Entomological indicators in the form of index measurement of *Aedes aegypti* larval density in particular settlements are essential considerations in effective vector control. **Table 3** shows that the HI was 23.89%, the CI was 7.81%, the BI was 50.73%, and ABJ was 76.10%.

The effect of the Entomology Index on dengue incidence in Bandung Municipality can be seen in **Table 4**. BRI is a factor that affects the elevation in dengue cases ($p = 0,001$). MI is a factor that influences the peak of dengue cases ($p = 0,001$). The presence of larvae is also a factor that causes an increase in dengue cases in Bandung municipality ($p = 0,000$). Meanwhile, the writer concludes that HRI could not be proven to influence the increase in dengue cases.

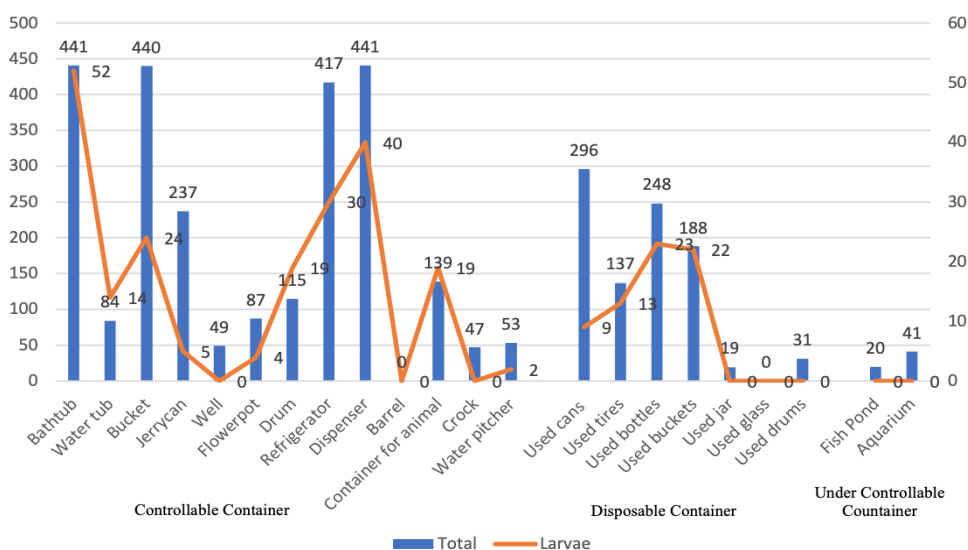


Fig. 2. Containers varieties on households in Bbandung municipality.

Table 2. The proportion of households based on the Breeding Risk Index (BRI), Hygiene Risk Index (HRI), and Maya Index (MI) in Bandung municipality

Category	BRI		HRI		MI	
	n	%	n	%	n	%
Low	428	78.7	491	90.3	420	77.2
Medium	7	1.3	7	1.3	83	15.3
High	109	20	46	8.5	41	7.5
Total	544	100	544	100	544	100

Table 3. Entomological Index

Entomological Index	Result, %	DF
House Index	23,89	4
Container Index	7,81	3
Breteau Index	50,73	6
Larvae Free Number	76,10	

Discussion

Common breeding sites observed in the study area were bathtubs, dispensers, refrigerators, barrels, and used plastic. Most residents in Bandung municipality store tap water and rainwater in containers for household necessities. Based on the research results, the incidence of dengue in Bandung City is quite inflated. This result is relevant to the data of dengue in Indonesia. Over a 50-year period, there has been a sharp increase in the annual IR of dengue in Indonesia, from only 0.05 cases per 100,000 person-years in 1968 to 77.96 cases per 100,000 person-years in 2016 [2]. The elevation of dengue cases in Bandung Municipality is reciprocal to the high number of mosquito larvae found. Thus, the

writer examined different containers in respondents' homes in Bandung Municipality and identified *Aedes aegypti* larvae and pupae. These findings are consistent with previous studies or research [16, 17]. A significant number of *Aedes aegypti* larvae were found, since the study site was in an urban area. Findings in Southern Taiwan show that *Aedes aegypti* almost exclusively exists in urban areas [18]. While the conclusions from a study in East Ethiopia from 405 containers were positive for mosquito larvae, 84.4% were identified as *Aedes aegypti* [19].

Nevertheless, in our research, *Aedes aegypti* was found to be the most dominant species to breed in artificial containers. Many containers are located near human habitation and are potentially more durable than natural containers [20]. In our research, no *Anopheles* mosquito larvae were found. Nonetheless, *Anopheles* mosquitoes inhabit old tires in Nicholas County, West Virginia [21]. The type of container, water quality, and condition of the water container are important for breeding³.

HI, CI, BI, and ABJ were relatively low. These findings are consistent and in line with the study that sta-

Table 4. The effect of Entomological Index on the incidence of dengue

Entomological Index	No Dengue		Dengue		Total		p-value
	n	%	n	%	n	%	
Breeding Risk Index (BRI)							0,001
low	235	54,9	193	45,1	428	100	
medium	6	85,7	1	14,3	7	100	
high	80	73,4	29	26,6	109	100	
Hygiene Risk index (HRI)							0,378
low	285	58,0	206	42,0	491	100	
medium	5	71,4	2	28,6	7	100	
high	31	67,4	15	32,6	46	100	
Maya Index (MI)							0,001
low	231	55,0	189	45,0	420	100	
medium	63	75,9	20	24,1	83	100	
high	27	65,9	14	34,1	41	100	
Presence of Larvae							0,000
absent	224	54,1	190	45,9	414	100	
present	97	74,6	33	25,3	130	100	

³ WHO. Chen C.D., Lee H.L., Stella-Wong S.P., et al. Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia; 2009. URL: <https://apps.who.int/iris/handle/10665/170721>

ted an overall HI of 14.2%, BI of 24.65, and CI of 5.9% [22]. All indices indicate a high level of risk of dengue transmission. A survey in Nepal found CI to be higher in the transitional period than in the rainy season [17]. A recent study in Malaysia conveyed maximum HI = 13,33%, BI = 13, dan CI = 19,05% [23]. In the literature, it has been criticized that this index needs more operational value and usage in assessing transmission risk. However, this index indicates a threat of global transmission at the community level [17]. The majority of *Aedes aegypti* larvae were found inside the respondents' homes. In accordance with findings in Southern Mexico, the frequency of larval populations was higher inside the households (14.1%) compared to containers found outdoors (5.1%) [24]. The writers found the most *Aedes aegypti* larvae in bathtubs, comparably with findings in Thailand that stated the most important risk factors for the existence of *Aedes aegypti* were container type (jars and tanks), location (toilet/bathroom), and lid status (no lid or covering) [25].

Amongst the risk factors for dengue incidence is the MI. In order to obtain the MI, the BRI and HRI were first calculated using the Miller formula [26]. The writers found that MI was in a low category. Another study in Tegal City stated that there was no difference between low and medium index MI categories. Thus, houses with low and medium index status have the equivalent risk of dengue infection [27]. The writers found that the most frequently controlled containers were bathtubs, buckets, jerry cans, refrigerators, dispensers, animal drinkers, and drums. Research in Semarang conveyed that mosquito larvae were primarily found in flowerpots and buckets, while those who suffered from dengue found the most mosquito larvae in bathtubs [28].

In this finding, BRI is in a low category, meaning that the danger as a mosquito breeding site is insufficient. This is consistent with other findings stating that the city is at low risk of mosquito breeding [29, 30]. Another research in Central Java found that BRI was at medium risk [31]. Several larvae were found in controlled containers, and all of them were *Aedes aegypti* larvae. The study in Eastern Ethiopia stated that the mosquito larvae-positive containers were mostly artificial water storage containers located outdoors (93.06%), open or partially covered (87.6%), and fully or partially exposed to sunlight (67.86%) [19].

The existence of mosquito larvae in the home can elevate the risk of dengue transmission. If there are mosquito larvae around the house, either indoors or outdoors, it will increase the risk of dengue infection. A study in Japan indicated that the highest number of wet containers were outdoors (56.5%), followed by indoors (32.2%) and roofs (11.3%). Among outdoor containers, 7.8% of containers were found to be overrun with *Aedes* larvae. Among indoor and rooftop containers, 3.1% and 3.9% of containers were found positive, respectively. The overall HI was 14.2. BI was 24.6,

and the CI was 5.9 [23]. All indexes showed that the transmission risk rate is currently high [32, 33]. So it is necessary to elevate efforts to prevent dengue by implementing mosquito nest eradication.

Conclusion

The study detected larval density based on entomological indices in the medium and high categories. BRI, MI, and larval occurrence can significantly elevate the incidence of dengue fever in Bandung Municipality. The writers suggest dengue vector control through routine inspection and destruction of potential container types both inside and outside the house as a preventive measure for dengue fever transmission.

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