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Water containers and the preferable conditions for laying eggs by *Aedes* mosquitoes in Maros Regency, South of Sulawesi, Indonesia

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ABSTRACT

One of the factors that influence the development of mosquitoes is the water container. This study was performed to determine the relationship between the characteristics of water containers and the preferable conditions for laying eggs by *Aedes* sp. A single larva method was conducted during March 2019 in 300 houses in two villages (Turikale and Adatongeng) at Maros Regency, Indonesia. In total, 1,269 water containers were considered, and among them buckets were found to be the most common container. Logistic regression analysis showed that the type, container location, and weekly drainage were related to the presence of larvae/pupae in the Maros Regency (p-value < 0.05). Non-water containers, which are kept indoors and are not drained at least once a week, have a greater chance of breeding *Aedes sp.* More attention is given to non-water containers that are located inside the house. Prevention activities, especially draining water containers properly at least once a week ensures that they do not become breeding sites for *Aedes sp.* mosquitoes and helps to prevent the transmission of dengue viral fever.

Key words: *Aedes sp*., container, Indonesia, mosquito, preference, water

HIGHLIGHTS

- Potential breeding sites for *Aedes sp*. at 1,269 water containers in 300 houses at Maros Regency, Indonesia were studied.
- Proportion of larvae/pupae was found the most in dispenser drip trays.
- Relationship between the type of water container, location, drainage frequency of the non-water containers and the presence of larvae/pupae of *Aedes sp*. (p-value < 0.05) was determined.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus, transmitted to humans through *Aedes aegypti* (*Ae. aegypti*) or *Aedes albopictus* (*Ae. albopictus*) mosquitoe bites (WHO 2009). The number of dengue cases has never decreased in some tropical and subtropical areas, and there is a tendency for the cases to continue to increase. The increase and spread of dengue cases are probably caused by high population mobility, urban development, climate change, population distribution, density, or other epidemiological factors that still require further research.

The World Health Organization (WHO) showed that 75% of the world's dengue cases between 2004 and 2010 were in the Asia Pacific. Indonesia was the country with the second-most prominent dengue cases among 30 endemic countries (Khori 2016). The morbidity rate of dengue fever in the last 5 years in Indonesia has fluctuated. In 2016, there was an increase in incidence rate (IR) compared to 2015, from 50.8 in 2015 to 78.9 per 100,000 population in 2016. There was a decline in 2017 and 2018 from 26.1 to 24.8 per 100,000. In 2019, the IR increased (51.5 per 100,000 population) and fell again in 2020 to 40 per 100,000 population. The problem that needs to be considered is the morbidity and mortality rate of DHF. In line with the number of cases, deaths due to DHF in 2020 also decreased compared to 2019, from 919 to 747 deaths. The case fatality rate (CFR) in Indonesia was 0.7% (MoH Indonesia 2021). DHF cases in South Sulawesi are 2,714 patients with morbidity of 29.6 per 100,000 population in 2020, which means there were 29–30 people with DHF in 100,000 population in South Sulawesi (CFR = 1.94%) (MoH South Sulawesi 2021). Based on the IR DHF, South Sulawesi is in the medium category (MoH Indonesia 2010), while the CFR is in the high category (MoH Indonesia 2021).

DHF is an environment-based disease influenced by human behavior, climate, and environmental conditions, resulting in the availability and accessibility of breeding sites for *Aedes sp*. as the vector (WHO 2009). ASEAN Dengue Day (ADD) launched the program 'Implementation 1 Home 1 Inspector (G1R1J)'. At the 2015 ADD, the G1R1J program was launched to reduce the morbidity and mortality of DHF by increasing the participation and empowerment of family-based communities for prevention. The direct support of the G1R1J program is the larva monitoring officer (JUMANTIK), who is a member of the community trained by the local health center to monitor the presence and development of mosquito larvae to control dengue disease in areas by eradicating mosquito breeding placess through the implementation of draining, burying, closing, followed by prevention of mosquito bites.

Breeding sites of *Aedes sp*. determine the presence of mosquitoes. One of the factors that influence the development of mosquitoes is water containers. The likelihood of Aedes sp. in laying eggs in a container is affected by the type, color, cover condition, location, and environmental conditions of the container, such as pH, temperature, and humidity of water (Daswito & Samosir 2021). In addition, the eradication behavior by determining the mosquito breeding places also affects the presence of larvae, such as draining water containers at least once a week (Azizah *et al.* 2018). So, it is necessary to conduct research determinants of the existence *of Aedes sp*. based on characteristics of the container, including type, color, location, cover, and behavior of draining water containers at least once a week in Maros Regency, which is an area that has carried out G1R1J counseling.

METHODS

The study was carried out in Maros Regency, South Sulawesi, Indonesia (centered between 40 °45′–50 °07′ south latitude and 109 °205′–129 °12′ east longitude). The research area covers two villages in Turikale District, Adatongeng and Turikale Village, which have received G1R1J counseling in Maros Regency.

The survey used a single larvae method for each water container containing larvae in March 2019. The study included 150 houses in each location (300 homes). Sampling was carried out at random houses at each site. The survey was carried out by checking natural and artificial containers inside and around the house and the presence of mosquito larvae. Water containers were inspected for the presence of mosquito larvae with a flashlight and a larva collection bottle was used for inspecting mosquito larvae. Data collection includes species, presence of mosquito larvae, lid conditions, colors, and container locations.

The types of container were divided into two categories: water containers and non-water containers. Water containers include bathtubs/toilets, buckets, basins/jars, drums, and other containers commonly used for collecting water, while non-water containers include bird waterer, dispenser drip trays, vases/flowerpots, goods used, and other containers that are not widely used for collecting water. Container colors were divided into two categories, dark (black, brown, gray) and light (red, yellow, blue, green) and similar color derivatives.

Larvae collected from the breeding sites were then identified to determine the species. Dissecting microscope and Leopolda M. Rueda identification picture key book for mosquito were used to identify the species of mosquitoes in the laboratory (Rueda 2004). All observations are recorded in the form/questionnaire collection.

Analysis was carried out to calculate the percentage of containers found with larvae/pupae based on type, color, location, lid condition, and drainage behavior. The proportion of infected ones calculated based on the containers found with larvae/pupae divided by the number of containers was observed. Factors related to larvae/pupae were obtained by chi-square analysis and continued with a logistic regression test using the SPSS version 18. The results of the analysis are presented in Tables 1–3. This study was carried out with ethical approval from the Health Research Ethics Committee, Health Research and Development Agency number: LB.02.01/2/KE.296/2018

RESULTS AND DISCUSSION

The observation of potential breeding sites for *Aedes sp*. was carried out in 300 houses in two villages (Turikale and Adatongeng) at Maros Regency, Indonesia. We inspected all water containers to check for the presence of larvae/pupae of *Aedes sp*. Based on the observations in each house we recorded the container types and calculated the proportion of infected containers. Details of water containers can be seen in Table 1.

Table 1 | The proportion of water containers infested with larvae and/or pupae in the two villages of the study area

Type of water containers/non-water containers	Turikale village			Adatongeng village			All village		
	Observed	Positive	Proportion infested (%)	Observed	Positive	Proportion infested (%)	Observed	Positive	Proportion infested (%)
Bathtub/toilet	23	1	4.3	112	27	24.1	135	28	20.7
Good used	27	2	7.4	26	6	23.1	53	8	15.1
Basins/jars	40	1	2.5	75	5	6.7	115	6	5.2
Drum	12	0	0.0	14	4	28.6	26	4	15.4
Bucket	399	3	0.8	410	22	5.4	809	25	3.1
Gallon	2	0	0.0	3	0	0.0	5	0	0.0
Jerry cans	1	0	0.0	1	1	100.0	2	1	50.0
Pool/Aquarium	16	0	0.0	7	1	14.3	23	1	4.3
Other	8	0	0.0	5	3	60.0	13	3	23.1
Dispenser drip trays	30	5	16.7	35	18	51.4	65	23	35.4
Waterways	1	0	0.0	3	0	0.0	4	0	0.0
Pet drinks	2	0	0.0	7	0	0.0	2	0	0.0
Vase/Flowerpot	8	0	0.0	2	0	0.0	7	0	0.0
Total	569	12	2.1	700	87	12.4	1,269	99	7.8

Table 2 | Conditions of containers with the presence of larvae/pupae in the study area

Village	Lid condition (%)		Color (%)		Position (%)		Drained once a week (%)	
	Open	Closed	Dark	Light	Outdoor	Indoor	Yes	No
Turikale Village	2.5	0.8	2.0	2.1	1.4	2.4	0.5	7.6
Adatongeng Village	14.6	5.4	14.0	12.1	10.3	13.3	1.5	28.1
All village	9.1	3.4	8.7	7.6	6.4	8.3	0.9	21.7

Table 3 | Analysis of water containers infested with larvae and/or pupae in the two villages of the study area

	Turikale village			Adatongeng village			All village		
	cOR (95% CI)	aOR (95% CI)	*p*-value	cOR (95% CI)	aOR (95% CI)	*p*-value	cOR (95% CI)	aOR (95% CI)	*p*-value
Type of water container (ref. water containers)	8.34 (2.58–26.94)	5.43 (1.37–21.54)	0.016	4.72 (2.78–7.99)	2.71 (1.46–5.02)	0.002	4.15 (2.65–6.51)	2.38 (1.40–4.07)	0.001
Lid condition (ref. close)	3.15 (0.40–24.63)	1.66 (0.17–15.77)	0.656	3.01 (1.47–6.14)	1.60 (0.72–3.54	0.224	2.82 (1.45–5.50)	1.74 (0.83–3.64)	0.137
Color (ref. light)	0.91 (0.19–4.23)	0.73 (0.14–3.80)	0.710	1.18 (0.67–2.06)	1.50 (0.78–2.90)	0.220	1.15 (0.68–1.92)	1.13 (0.63–2.02)	0.666
Position (ref. outdoor)	1.77 (0.38–8.20)	7.93 (1.47–42.71)	0.016	1.33 (0.78–2.27)	3.19 (1.71–5.94)	0.000	1.32 (0.81–2.16)	3.70 (2.10–6.53)	0.000
Drained once a week (ref. Yes)	17.82 (2.85–82.44)	18.91 (3.81–93.82)	0.000	26.47 (11.36–61.70)	25.85 (10.87–61.45)	0.000	29.07 (13.95–60.57)	30.52 (14.44–64.52)	0.000

A total of 1,269 water containers was observed in this study. Table 1 shows that buckets were the most commonly observed container type in both villages (399 in Turikale and 410 in Adatongeng). Still, the proportion of larvae/pupae was found the most in dispenser drip trays and used goods containers in Turikale Village. In contrast, the highest proportion was found in jerry cans and dispenser drip trays in Adatongeng.

Indonesian people have a habit of storing water for daily needs such as drinking, cooking, and bathing, so every house has plenty of water containers. The limited amount of clean water often causes residents to store large quantities of water in containers for their daily needs (Kinansi & Pujiyanti 2020). This habit potentially causes the number of water storage containers in each house to become breeding sites for mosquitoes. In this study, we found that the average container in the place ranged from 3 to 6 containers per house (Adatongeng = 4.6 and Turikale = 3.8). This study found buckets to be the most common type of container, followed by bathtubs/toilets. Although located in large numbers, there were not many larvae in the bucket because the amount of water in the bucket was not too large, so people often used it. This situation does not match the habit of mosquitoes that like to lay their eggs in stagnant water. Previous research also showed that large containers with water volume >50 L produce 4.9 times more pupae than containers with <1 L (Islam *et al.* 2019). This fact explains that although the number of buckets in the house is enormous, there are very few *Aedes sp*. at the location.

Based on Table 1, the type of container with the most *Aedes sp*. is jerry cans (50%), followed by dispenser drip trays (35.4%) and bathtubs/toilets (20.7%). Homeowners are often unaware of stagnant water in secret places like jerry cans and dispenser drip trays that enable mosquitoes to lay their eggs in these locations. The water collected in the dispenser drip trays is accidentally caught. The container is closed but has holes allowing mosquitoes to lay eggs easily because its location is hidden and often goes unnoticed (Prasetyowati *et al.* 2018). Some people also do not know that the dispenser drip trays are the containers that are often the breeding sites for mosquitoes (Hendayani *et al.* 2022). Containers that store water for a long time are good places for developing *Aedes sp*. This must be a concern because this type of container is rarely noticed during larva inspection by households or larvae monitors. The larvae on the dispenser drip trays are also found in Bandar Lampung (Nurjanah *et al.* 2021).

In this study, we also found that the bathtub/toilet to be the main container for the development of *Aedes sp*. Indonesians have a habit of storing water for bathing. Usually, water is stored for a long time. If the community does not regularly drain and replace water, then the place becomes a potential breeding site. The activities of draining and cleaning bathtubs are often not routinely carried out by residents. Emptying and cleaning the bathtub is usually only done when residents have free time. The habit of residents who immediately add to the contents of the tub when the amount of water is low without draining and cleaning the tub causes the number of larvae of *Aedes sp*. to increase more and more (Utami & Haqi 2018). The average bathtubs/toilet cannot be closed tightly due to practical problems when using the water, allowing *Aedes sp* mosquitoes to lay their eggs in the dispenser drip trays easily (Kinansi & Pujiyanti 2020). Based on previous studies, water stored for a long time, prolonged rain, humidity, and appropriate temperature can support the proliferation of *Aedes* sp. (Abílio *et al.* 2018). This has also resulted in some countries using showers instead of bathtubs for bathing. The bathtub is one of the containers that can potentially be the breeding sites for *Ae. aegypti*, because the bathtub is used for daily activities, and filling the tub with water always becomes habitual. Also, the condition of the bathtub being kept open or unclosed; having a large size; and the location of the tub being indoors, so this situation will protect it from direct sunlight, and the air tends to be more humid. The condition of the bathtub supports the egg-laying behavior of *Ae. aegypti*, as it prefers breeding places with high humidity, dark, moist, stagnant clear water, and wide-open surface. The large size is also one of the reasons why residents are reluctant to clean the water tank, therefore, microorganisms grow on the walls of the water containers and become a source of food for larvae (Azizah *et al.* 2018). The surface of the bathtub/toilet is usually made of cement or ceramic, and the cover is cooler so that food sources for larvae such as algae, plankton, and fungi grow easily (Kinansi & Pujiyanti 2020).

The container type is related to the presence of Aedes sp. (p-value < 0.05). Non-water containers are not used for daily needs, so they often escape the attention of homeowners. Non-water containers, including bird waterer, dispenser drip tray, vases/flowerpots, used goods, and other containers that are not commonly used as water containers, have a greater chance of containing mosquito larvae (OR = 2.38; 95% CI: 1.40–4.07) compared to water containers such as bathtubs/toilet, buckets, basins/jars, drums, and other containers commonly used as water containers. Similar results were obtained from studies in Tanjung Seneng, Bandar Lampung, and Tanjung Pinang (Daswito & Samosir 2021; Nurjanah *et al.* 2021).

We also observed the condition, color, position, and drainage behavior. A summary of containers with the presence of larvae/pupae can be seen in Table 2. Table 2 shows that larvae/pupae were more commonly found in open water containers that are dark in color, kept indoors, and are not drained once a week.

Logistic regression analysis showed that type, container location, and drainage behavior were related to larvae/pupae in the villages of Turikale and Adatongeng (p-value $<$0.050). Non-water containers kept indoors and not drained once a week have a greater chance of breeding *Aedes sp*. Detailed results of the analysis are seen in Table 3.

The results showed that many larvae were found in open containers. The open container allows female *Aedes sp*. to lay eggs on the surface of the container. *Aedes sp*. habitually lays eggs slightly above the water's surface. Although, statistically, there was no relationship in this study between the condition of open/closed containers and the presence of larvae (p-value $>$ 0.050), this is possible because other characteristics of the container affect the presence of larvae. Results of this study are similar to the results in research works by Sukarame and Kemiling, but in Tanjung Seneng, Bandar Lampung City, it shows the opposite (Nurjanah *et al.* 2021), the same as in Tanjung Pinang Timur (Daswito & Samosir 2021).

Dark-colored containers are more likely to increase the number of larvae because the *Aedes sp*. likes dark places. Mosquitoes tend to lay more eggs in dark-colored containers because mosquitoes feel safer and calmer laying eggs in dark places (Kumawat *et al.* 2014; Madzlan *et al.* 2016). We found more dark containers, consistent with previous research, which showed that the *Aedes sp*. prefers to lay its eggs in a dark-colored container rather than a light-colored one (Nurjana & Kurniawan 2017; Tsunoda *et al.* 2020) However, statistically, there is no relationship between container color and the presence of larvae (p-value $>$ 0.05). The results of this study are in line with the survey in Sukarame Bandar Lampung, but in other locations, it shows a relationship (Nurjanah *et al.* 2021).

Ae. aegypti prefers clean water in containers inside and around the house, while *Ae. albopictus* is more likely to be found in natural containers or outdoor artificial habitats, where organic waste is abundant. The location of water containers for daily needs is usually placed in a part of the house that is not exposed to direct sunlight, tends to be damp and calm, and is rarely cleaned (Kinansi & Pujiyanti 2020). Statistically, it shows a relationship between the location of containers inside/outside the house and the presence of larvae in Turikale and Adatongeng villages. Containers inside the house are 5.43 and 2.71 times more likely to contain larvae than containers outside the home. This study is consistent with the research results in Tanjung Pinang (Daswito & Samosir 2021), however, studies in Bandar Lampung City at three locations and Colombia show the opposite (Nurjanah *et al.* 2021).

This study showed that mosquito larvae were more commonly found in containers that were not drained once a week. Draining water can inhibit the development of *Aedes sp*. The lifecycle of mosquitoes starting from eggs to adults takes an average of 9 days, so emptying water containers once a week can break the development cycle of *Aedes sp*. The activity of draining the water, including cleaning the walls, removes mosquito eggs that are still attached to the walls of the water containers. Draining without cleaning and using soap does not remove the eggs stuck to the walls of the water containers (Azizah *et al.* 2018). The practice of draining can increase the presence of larvae in some places if cleaning/draining has not become a continuous habit, using improper draining technique, or draining after more than a week. The water containers should be emptied at least once a week, scrubbing the walls and dousing them with hot water to completely release the eggs attached to the walls of the water containers (Kinansi *et al.* 2019). Statistically, the behavior of draining water containers at least once a week is related to the presence of larvae (p-value $<$ 0.05), in line with research in Jonggol District, Bogor Regency (Azizah *et al.* 2018). A study in Colombia showed that containers were washed monthly and had risk of finding larvae at 4.23 and 4.55, respectively, compared to containers washed weekly (Overgaard *et al.* 2017).

There seem to be relations between the type of water container, location, drainage frequency of the non-water containers, and the presence of larvae/pupae of *Aedes sp*. (p-value $<$ 0.05). More attention was given to the type of non-water containers located indoors, especially to secret places where mosquitoes breed. Prevention activities include draining water containers properly once a week so they do not become breeding sites for *Aedes sp*. mosquitoes that act as carriers for dengue fever.

CONCLUSIONS

There seems to be a link between the type of water container, location, drainage frequency of the non-water containers, and the presence of larvae/pupae of *Aedes* sp. (p-value $<$ 0.05). More attention was paid to non-water container types that were located indoors, especially to secret places where mosquitoes would breed. Prevention activities include draining the water containers properly once a week so that they do not become breeding sites for *Aedes sp*. mosquitoes that act as the carriers for dengue fever.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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