



Aedes Aegypti Hatchability and Larval Development Based on Three Different Types of Water

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ABSTRACT

Clean water reservoirs can become mosquito breeding grounds because water comes from rainwater, water wells, and taps, making them an ideal breeding ground for the *Aedes aegypti* mosquito vector of the dengue virus. This study aims to determine the hatchability of eggs and the growth of *Aedes aegypti* in three different types of brooding water in Sako Village, Palembang City. This study is experimental. The sample contains 1200 *Aedes aegypti* eggs collected from the Health Research and Development Center (Balitbangkes), the Ministry of Health of Indonesia in Baturaja, as well as three types of water collected from the homes of residents suffering from Dengue Hemorrhagic Fever in Sako Village, Sako District, Palembang City. Data was collected through direct observation for 5 days of egg hatching in the third water, which was repeated three times. The study's results were analyzed by the ANOVA, Honest Significant Difference test (BNJ), and R studio software version 4.1.2. The average number of mosquito eggs that hatched in well water was 77.67%, rainwater was 63.33%, tap water was 54.67%, and control was 72.33%. There were significant differences in the effects of the three types of air on the larval and pupal stages of *Aedes aegypti* development. This study shows that *Aedes aegypti* mosquitos can breed into adults outside of clean water and water that contains soil, such as water wells.

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ABSTRAK

Tempat penampungan air bersih dapat menjadi sarang nyamuk, air berasal dapat berasal dari air hujan, sumur air dan ledeng sehingga menyediakan tempat yang baik bagi nyamuk *Aedes aegypti* vektor virus dengue untuk berkembang biak. Penelitian ini bertujuan untuk mengetahui daya tetas telur dan perkembangan *Aedes aegypti* pada tiga jenis air perindukan di Kelurahan Sako Kota Palembang. Penelitian ini merupakan penelitian eksperimental. Sampel terdiri dari 1200 butir telur *Aedes aegypti* yang diperoleh dari Litbangkes Kemenkes Baturaja dan tiga jenis air yang diambil dari rumah warga yang menderita penyakit Demam Berdarah Dengue yang bertempat tinggal dan menetap di Kelurahan Sako, Kecamatan Sako, Kota Palembang. Pengumpulan data diperoleh dengan melakukan pengamatan langsung selama lima hari terhadap penetasan telur pada ketiga air tersebut yang dilakukan sebanyak tiga kali pengulangan. Hasil penelitian di analisis menggunakan ANOVA, uji Beda Nyata Jujur (BNJ) dan *software* R studio version 4.1.2. Hasil penelitian menunjukkan rata-rata jumlah telur nyamuk yang menetas di air sumur mencapai 77,67%, air hujan 63,335, dan air PDAM 54,67% dan kontrol sebesar 72,33%. Terdapat perbedaan yang signifikan pengaruh tiga jenis air terhadap perkembangan stadia larva dan pupa *Aedes aegypti*. Penelitian ini membuktikan bahwa nyamuk *Aedes aegypti* mampu berkembang biak menjadi dewasa di luar media air bersih dan air yang dasarnya mengandung tanah seperti air sumur.

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is an acute epidemic disease spread by mosquitos carrying the dengue virus, *Aedes aegypti*, and *Aedes albopictus* (Wang et al., 2020). Dengue fever is still a health issue in Indonesia, where the number of sufferers is increasing and the spread is becoming more widespread. Indonesia ranks second among 30 endemic countries in terms of dengue cases. There were 68,407 cases reported, with 493 deaths (Harapan et al., 2019)(Haryanto, 2018). In 2018, there were 2,437 DHF cases in South Sumatra Province, with 26 deaths. Palembang City, with 620 cases, has the highest number of cases in South Sumatra Province. In 2019, there were 667 DHF cases in Palembang city, 435 cases in 2020, and 246 cases in 2021(Dinas Kesehatan Provinsi Sumatera Selatan, 2022).

The main breeding sites for *Aedes aegypti* are areas with clean water near people's homes. In general, people use clean water for their daily needs, which can be water from a drinking water company, rainwater, and well water. These three types of water have slightly different characteristics and are intentionally or unintentionally accommodated, resulting in mosquito breeding grounds (McNaughton et al., 2018). Well, water has low salinity, organic matter, neutral pH, clear or low turbidity, and is abundant, making it ideal for the life of *Aedes aegypti* (Cahyati & Siyam, 2019).

Several previous studies on the effect of water type on *Aedes aegypti* hatchability found that dug well water had the highest hatchery, at 34.45% % (Cahyati & Siyam, 2019). According to research conducted in Kelurahan Kebun Bunga, Sukarami District, Palembang City, well water has the highest number of hatches (26.66%), while tap water has the lowest (2.7%) (Lestari et al., 2021). The findings of this study are consistent with previous research into the most preferred breeding locations for *Aedes aegypti* females based on the type of water source, indicating that *Aedes aegypti* prefers to lay eggs in dug well water (Ningsih et al., 2016). According to (Mawardi & Busra, 2019) Because the well water is clear and clean, it attracts *Aedes aegypti* mosquitos to lay their eggs. Microorganisms found in well water provide food for mosquitos. Meanwhile, chlorine ($\text{Ca}(\text{OCl})_2$) is a disinfectant found in tap water. Other studies have shown that several active substances in water can affect the hatchability of mosquito eggs. The presence of chlorine in chlorine, which is capable of oxidizing the eggs of *Aedes aegypti*, can interfere with the hatching process when used in water media. The lower the hatchability of *Aedes aegypti*, the higher the chlorine concentration in the water (Agustin et al., 2017) (Irwinsyah et al., 2018).

Environmental control, such as preventing the formation of breeding sites or habitats for mosquitoes, is one method of mechanically eradicating the DHF vector. This study aims to determine the hatchability of *Aedes aegypti* in three different types of water, inform the public about potential breeding sites for *Aedes aegypti*, and take action to prevent and eradicate breeding sites.

METHOD

This is an experimental study that has a completely randomized design. The research was carried out at the Laboratory of Parasitology, Faculty of Medicine, Muhammadiyah University, Palembang, from August to December 2022 *Aedes aegypti* Liverpool strain was obtained

from Litbangkes Kemenkes Baturaja rearing with the same age to ensure sample homogeneity. Water samples include well water, rainwater, and tap water, all of which are used by Indonesians. A positive control using Aquades distilled water as a positive control. Wells water, rainwater, and tap water are obtained from the homes of Dengue Hemorrhagic Fever patients living in Sako Village, Sako District, Palembang City. Water Municipal Waterworks is taken directly from the tap without settling, rainwater is taken from water that falls when it rains and is accompanied by stagnant water in used goods, and well water is taken from dug wells.

Sources of well water, rainwater, and tap water in Palembang City's Sako sub-district, the Sako Health Center area, because it is a DHF endemic area with the highest DHF incidence rate in 2019 (Dinkes Provinsi Sumsel, 2020).

Procedures

Aedes aegypti mosquito eggs were removed one at a time from the filter paper, and 100 eggs were placed in each container containing 250 ml of different water and covered with gauze. This process was repeated three times, and the eggs were observed and recorded for five days. Eggs that hatch into larvae are transferred to a 26x21x4cm³ plastic tray containing 750 L of water, the larvae begin to be fed dog pellets as much as 20 mg per day at the age of two days, and the larvae will turn into pupae within one week, the larvae that have become pupae are transferred one by one using a pipette into a large volume plastic cup 250 mL filled with 1/4 of the water, a plastic cup (Ramayanti et al., 2023).

The hatchability of mosquito eggs was calculated by dividing the total number of eggs hatched in each type of water by the total number of egg samples. Water pH was measured with a pH meter (accuracy level of 0.1) once for each type of water. To ensure homogeneous environmental conditions during research, daily measurements of room temperature and humidity are taken with a digital thermometer hygrometer at 08.00, 12.00, and 16.00.

Data analysis

The hatchability of *Aedes aegypti*, water pH, room temperature, and humidity were all variables in this study. The obtained data were analyzed using one-way ANOVA and then the Honest Significant Difference (BNJ) test with a 5% level of confidence in significantly different results. All data were tabulated and analyzed using R studio version 4.1.2 software.

RESULTS AND DISCUSSION

Aedes aegypti Egg Hatchability

Table 1 shows the number of eggs of *Aedes aegypti* that hatched in three types of brooding water and one control after five days of observation.

In this study, it was discovered that well water produced significantly different results than other types of water. It was discovered that well water had the highest percentage of hatched eggs when compared to other types of water.

Table 1
Aedes aegypti egg hatchability Water classification of Water type

Treatment of Water Type	Hatching rate (%)
Aquades	72.33 ^a
Well water	77.67 ^a
Rainwater	63.33 ^b
Tap water	54.67 ^c
F-Count	43.12 ^{**}
P-Value	2.77×10 ⁻⁵
BNJ 5%	4.29

ns = not significantly different; * = significantly different; ** = significantly different real. The highest number is a the second highest number is b, and the lowest number is ab/c. At the 5% BNJ test level, numbers followed by the same letter in the same column are not significantly different; the original data is transformed first using the arcsin transformation during data processing.

Table 2
The mortality rates of larvae and pupae.

Treatment	Larval mortality rate (%)	Pupae mortality rate (%)
Aquades	0.33 ^c	0.00 ^b
Well water	0.33 ^c	0.00 ^b
Rainwater	10.33 ^b	1.00 ^{ab}
Tap water	36.33 ^a	2.67 ^a
F-Count	85.14 ^{**}	11.07 ^{**}
P-Value	2.07×10 ⁻⁶	3.22×10 ⁻³
BNJ 5%	8.22	6.04

ns = not significantly different; * = significantly different; ** = significantly different real. The highest number is a the second highest number is b, and the lowest number is ab/c. At the 5% BNJ test level, numbers followed by the same letter in the same column are not significantly different; the original data is transformed first using the arcsin transformation during data processing.

Table 3
The differences in the long development time of *Aedes aegypti* stadia treated with various water sources.

Treatment	Long stadiums (days)					
	The eggs hatch	1st instar	2nd instar	3rd instar	4th instars	Pupae
Aquades	5.00	4.00	6.00 ^a	4.00 ^a	3.00 ^a	3.00
Well water	4.00	4.00	2.00 ^c	5.00 ^b	2.00 ^b	3.67
Rainwater	5.00	4.00	5.00 ^b	4.00 ^a	3.00 ^a	3.67
Municipal Waterworks	5.00	4.00	5.00 ^b	4.00 ^a	3.00 ^a	4.00
F-Count	1.8 ^{ns}	1.00 ^{ns}	3.84 ^{**}	3.81 ^{**}	2.27 ^{**}	3.17 ^{ns}
P-Value	0.22	0.44	2.00×10 ⁻¹⁶	2.00×10 ⁻¹⁶	2.00×10 ⁻¹⁶	0.09
BNJ 5%	-	-	9.91	2.67	1.38	-

ns = not significantly different; * = significantly different; ** = significantly different real. The highest number is a the second highest number is b, and the lowest number is ab/c. At the 5% BNJ test level, numbers followed by the same letter in the same column are not significantly different; the original data is transformed first using the arcsin transformation during data processing.

Factors of the Environment (Water pH, Room Temperature, and Humidity)

The following environmental factors were observed during the hatchability and development of the aquatic stages of *Aedes aegypti* in this egg hatchability study: pH, temperature, and humidity.

Table 4 shows the results of water pH measurements for each water medium. As a control, the highest water pH was found in distilled water, at 7.72, and the lowest in tap water, at 6.48. The temperature and humidity in the room are kept at optimal levels for the development of *Aedes aegypti*.

The effect of water type on *Aedes aegypti* larvae, and pupae mortality

The goal of observing the mortality of *Aedes aegypti* larvae and pupae is to determine the effect of the three types of water used on mortality during the developmental stages of *Aedes aegypti*. Table 2 shows the percentage of mosquito *Aedes aegypti* larvae and pupae mortality. When compared to well water and rainwater, the results obtained were very significantly different in the treatment using tap water, which caused the highest mortality.

***Aedes aegypti* mosquito aquatic development stadia**

The findings of observations on the length of time it takes for eggs to hatch and for larval and pupal stages to develop in three types of water and one control.

The length of time the eggs take to hatch and the stages of aquatic development of *Aedes aegypti* were also observed. The first instar eggs and larvae produced no significantly different results when hatching. In the following stage, the 2nd instar larvae produced significantly different results: it took 2 days for development in well water, 5 days in rainwater and Regional Drinking Water Company water, and 6 days in control water. When compared to other water treatments, the development of instar 3 larvae in well water produced significantly different results. In contrast, 4th instar larvae produced significantly different results in well water after only 2 days of development. Observing the development of the pupae revealed that the pupal stage in all treatments lasted 3-4 days.

Table 4
The pH of water, room temperature, and humidity.

Type of Water	pH	Temperature average	Humidity average
Aquades	7,72		
Well water	7,6	20.7°C	58.8%
Rainwater	7,1		
Tap water	6,48		

DISCUSSION

In this study, *Aedes aegypti* eggs can hatch in all types of water, with the most hatching occurring in well water, followed by rainwater, and tap water, and there are significant differences in the hatchability of *Aedes aegypti* to the three types of water. These findings are similar to (Lestari et al., 2021) research in Kelurahan Kebun Bunga, Sukarami District, Palembang City, which discovered that all *Aedes aegypti* eggs could hatch in all three types of water, with well water serving as the most important hatchery for *Aedes aegypti*. (Mataram & Warni, 2017) discovered that the hatchability of *Aedes aegypti* is higher in well water than in sewer water and that the type of water has an effect on egg hatchability. *Aedes aegypti* was discovered in nine of ten wells studied in Queensland, Australia (Trewin et al., 2017). This indicates that wells are a potential habitat for *Aedes aegypti* because well water is not cloudy and has a low chemical content.

The well water is a potential habitat for *Aedes aegypti* because it has a high level of salinity and a low level of alum, resulting in high dissolved oxygen. The many chemical constituents and the nature of water from well water are also favorable to *Aedes aegypti*. Female mosquitoes laying eggs are drawn to the characteristics of well water (Ratnasari et al., 2021).

When compared to other types of water, larvae, and pupae of *Aedes aegypti* obtained from tap water had the highest larval and pupal mortality. This is suspected to have chemicals in the water that affect the hatchability of *Aedes aegypti* eggs. The research (Agus & Gazali, 2020) discovered that the chlorine content in the water media could interfere with the development and hatching processes of *Aedes aegypti*. The higher chlorine concentration indicates that chlorine is toxic to *Aedes aegypti* mosquito eggs. Because chlorine is a disinfectant that can reduce the amount of oxygen dissolved in water, the concentration of chlorine in the water can inhibit the development of mosquito eggs (Irwinsyah et al., 2018). This is supported by dissolved oxygen analysis using the method Winkler demonstrated that the high concentration of chlorine dissolved oxygen was the lowest (Ikawati & Meilani, 2017).

The outcomes of watching *Aedes aegypti* larvae develop into pupae in various types of water. All types of water have different effects on the larval stage's long stages of development. According to the findings of (Jacob et al., 2014) research, *Aedes aegypti* larvae can not only survive in clear water but also in sewage water that is still and clear. *Aedes aegypti* mosquito growth and development are aided by a variety of environmental factors, including physical, chemical, and biological conditions. This is also supported by mosquitoes' ability to adapt to their surroundings, making them very resilient and able to recover quickly after disturbances caused by natural phenomena (Ratnasari et al., 2021). *Aedes aegypti* breeds normally in clean standing water that is not in direct contact with the ground (Marza & Shodikin, 2016).

The degree of acidity or pH, temperature, humidity, light, oxygen content, chemicals in the water, and the condition of the egg itself are all factors that can influence the hatching of *Aedes aegypti* (age, eggshell). In this study, dug well water has the highest pH and the greatest hatchability of mosquito eggs, which is consistent with the findings of (Suparyati & Himam, 2021), who found that dug well water has the highest hatching rate of *Aedes aegypti* with a pH of 7.56. Rainwater and tap water from municipal waterworks, as well as controls, have a pH lower than dug well water and have a low hatchability of mosquito eggs. Thus, the lower the acidity or

pH, the lower the hatchability of mosquito eggs. These results are from previous studies which showed that the lower the pH of the brooding water (acid), the decrease in mosquito acquisition was more significant than the increase in the pH value (Suparyati & Himam, 2021). A pH of 4-9 is ideal for mosquito egg hatching. The pH of the water affects the levels of oxygen and carbon monoxide, which in turn affects the formation of *Aedes aegypti* and *Aedes albopictus* (Cahyati & Siyam, 2019).

Aside from the pH factor, which influences the hatchability of mosquito eggs. Temperature influences the mosquito breeding cycle during the egg, larva, and pupa stages, including virus development in the mosquito's body, biting rate, resting and mating behavior, and the spread and duration of the gonotrophic cycle (Putri et al., 2020). Mosquitoes will lay their eggs at temperatures ranging from 20 to 30 °Celsius (Baskoro et al., 2017). The optimal temperature for *Aedes aegypti* hatching is 25-27°C. Mosquitoes grow shorter and reproduce faster as temperatures rise due to climate change, meanwhile, *Aedes aegypti* eggs will not hatch if the temperature is between 10-15°C (Reinhold et al., 2018). According to the findings of this study, all types of water were treated at room temperature, with an average temperature of 20°C, indicating that the temperature in this study was optimal for hatching *Aedes aegypti*.

Humidity is also an important factor in *Aedes aegypti* hatching. *Aedes aegypti* can generally survive in moist air that is neither too dry nor too humid (Reinhold et al., 2018). Mosquito larvae can survive in humidity levels that are appropriate for air temperatures that are neither too cold nor too hot. The optimal humidity for the life cycle of *Aedes aegypti* is around 81.5-89.5%, whereas *Aedes aegypti* will have a short life if humidity is low, which will affect evaporation in mosquitoes, thereby shortening their life. When exposed to water, 7 days will hatch immediately (Rasjid et al., 2019).

The temperature and humidity of the water, as well as the environment, are major factors influencing egg hatching. Mosquitoes, for example, are affected by humidity and temperature. Mosquitoes cannot complete their life cycle at certain temperatures and humidity levels. At a specific temperature, perfect mosquito morphology (eggs, larvae, pupae, and adults) can carry out optimal development (CDC, 2016). Temperature and the area of its habitat influence each mosquito's egg phase in the process of hatching eggs optimally. The physiological growth of *Aedes aegypti* is influenced by temperature (Reinhold et al., 2018).

The findings of this study indicate that *Aedes aegypti* can adapt to an unfavorable environment, so when pressed by mosquitoes, they can lay their eggs in an unfavorable location to survive. Dengue Hemorrhagic Fever Eradication includes all efforts to prevent and treat DHF, as well as actions to limit disease spread. The eradication of mosquito nests program is used to limit and prevent DHF cases through draining, burying, covering, larviciding, keeping fish activities, and other methods used to eradicate larvae (Kemenkes RI., 2017).

LIMITATIONS OF THE STUDY

The study's limitation is that it only examines the effect of chlorine concentration on the hatchability of *Aedes aegypti* eggs.

CONCLUSIONS AND RECOMMENDATION

The hatchability of *Aedes aegypti* differs in three types of water, namely well water, rainwater, and tap water, with well water being the best hatching medium. Efforts to control dengue disease in the form of eradicating *Aedes aegypti* mosquito nests from all types of breeding places, as well as paying attention to the cleanliness of the surrounding environment and stagnant water around households and public places, so that it does not have the potential to become a breeding ground for *Aedes aegypti* mosquitoes, which have the potential to be DHF vectors. The 3M Plus program must be implemented on an ongoing basis throughout the year, particularly during the rainy season.

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Conflict of Interest statement

The authors declare no conflict of interest.

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