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Description of the Behavior of *Aedes aegypti* Mosquito Laying Eggs in Water Contaminated with Animal Feces and Soil

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ABSTRACT

Background & Objective This study aims to determine the egg-laying behavior of *Aedes aegypti* in water contaminated with animal waste and soil, count eggs caught in cages, observe and count the number of healthy and damaged eggs, and compare the number of eggs from each water medium.
Method This study is descriptive, the data were analyzed using the SPSS application, presented in tabular form, and explained in a narrative manner.
Result The results of observations of egg-laying behavior indicate that water contaminated with animal waste and soil can be a breeding ground for the *Aedes aegypti*.
Conclusion Based on the results of observations of the behavior of *Aedes aegypti* in water media contaminated with animal feces and soil, it shows a distinctive behavior, that water contaminated with animal feces and soil is favored by *Aedes aegypti* as a place to lay eggs and breed. The total number of eggs caught was 1,716, with the number of healthy eggs (1,219) and the number of damaged eggs (497). Differences in the content contained in the water media affected the laying of *Aedes aegypti* eggs, but did not affect the number of healthy or damaged eggs.

Keywords *Aedes aegypti*; Dengue Hemorrhagic Fever; environmental conditions; behavior.

Introduction

Aedes aegypti is a vector spreading dengue fever (DHF) is not a new disease in Indonesia, it has been known since 1779. Dengue hemorrhagic fever is a public health problem in Indonesia. Dengue hemorrhagic fever (DHF) is a rapid infectious disease because the dengue pathogen is a dengue virus that enters the human body through bites. In endemic areas, an increase in dengue cases can occur in a short time and can even cause abnormal events (KLB) (Syamsir & Pangestuty, 2020).

According to WHO, several countries in the Western Pacific, Southeast Asia and the Americas have the largest number of dengue patients. The number of recorded cases exceeded 1 million in 2008 and then increased to 3 million in 2015. In 2016 there were outbreaks of dengue fever around the world, especially in equatorial countries including Indonesia. In 2015, Indonesia reported 129,650 cases of dengue fever, of which 1,071 people died, compared to 100,347 cases in 2014, this figure shows an increase in the number of cases (Syamsir & Pangestuty, 2020). Director of the Department of Prevention and Control of Vector and Zoonotic Infectious Diseases. Siti Nadia Tarmizi, M. Epid confirmed that until July 2020, the number of Dengue Hemorrhagic Fever (DHF) cases in Indonesia reached 71,633. The 10 provinces with the highest number of reported cases in West Java with 10,772 cases (Kasenda et al., 2020). Studying behavior is important to us, because it is one of the vector control strategies. There are no vaccines or drugs recommended for treatment and prevention. DHF is the reason why vector control is carried out (Syamsir & Pangestuty, 2020).

Previous research conducted by Makna Fathana Sabila (2013) used well water, chlorine, and straw bath water to facilitate egg laying. Research on the mosquito *Ae. Aegypti* has previously been conducted by Makna Fathana Sabila (2013) using well water, chlorine, and straw water to see egg laying preferences. The process of choosing a place is influenced by organic matter and ammonia. Media contaminated with animal feces and soil contain higher levels of organic matter and ammonia, so mosquitoes are more interested in breeding in these media. High organic matter allows the media to have sufficient food for the survival and growth of offspring (Sabila et al., 2013). Media contaminated with animal feces and soil contains higher levels of organic matter and ammonia, so are more interested in breeding in these media. High organic matter allows the medium to have sufficient feed for the survival and growth of offspring (Sabila et al., 2013).

All need water for their life cycle. are insects that are very capable of utilizing water and artificial sources both permanent and temporary. The life cycle is strongly influenced by the availability of water as a breeding medium from eggs to adult es. survival requires three places, namely breeding grounds, blood-sucking grounds and habitats. These three places are closely related to survival (Supriyono et al., 2019).

Allah Almighty said in Q.S An-Nur verse 45 which means "And Allah has created all kinds of animals from water, then some of the animals are walking on their stomachs and some are walking on two legs while some (others) are walking on four legs. God created what He willed, indeed God is almighty over all things".

In the Quranic verse, it does not specifically explain es, but the verse provides information that Allah Almighty has created several types of aquatic animals, which means a truth that water is a breeding ground for living things, both large and small living things such as es. Where I need a water medium to lay eggs. Indeed, Allah Almighty is almighty with all things.

There are more than 2500 species in the world, one of which is *Aedes aegypti*. *Aedes aegypti* prefer dark breeding grounds, which can be protected from the sun both inside and outside the house. Theoretically explains the *Aedes aegypti* have breeding grounds in clear water, such as bathrooms, flower pots, pet drinking places, and old things that are submerged in water, accommodated in containers and do not directly touch the ground. However, some studies show that the reproductive behavior of has changed.

Aedes Aegypti can breed and live in chlorine-containing water, and can lay eggs in horse and cow manure. Hence the *Aedes aegypti* shows behavioral changes that adapt to the environment (Agustin et al., 2017).

The existence of the *Aedes aegypti* vector can be influenced by biotic or abiotic environmental factors ranging from the egg stage to the adult stage. Abiotic factors such as precipitation, temperature and evaporation. Similarly, biological factors, such as predators, competitors, and food breeding grounds, including organic matter, microorganisms, and aquatic insects, can affect the survival of before they reach adulthood. Based on these problems, the author feels it is important to conduct research to determine the effectiveness of broiler chicken manure water, pig manure and soil contaminated water media, considering that in these three media there are organic substances and ammonia that are high enough to be able to attract *Aedes aegypti* for laying eggs. The author also feels it is important to know the number of healthy and damaged eggs and see how Ae lay becomes. aegypti in a water medium contaminated with animal feces and soil. If Aedes aegypti can breed in various water media, so the potential danger of transmitting dengue or other diseases is greater, because more and more places are their living habitat (Agustin et al., 2017).

Objective

This study aims to determine the Description of Egg laying Behavior of *Aedes aegypti* in Water Contaminated with Animal Manure and Soil. Aspects of egg behavior in this study include the egg-laying behavior of Ae es. aegypti on the water medium contaminated with animal feces and soil, counting the number of eggs of *Aedes aegypti* caught inside ovitrap, water media contaminated with animal feces and soil is preferred or not as a place to lay eggs for *Aedes aegypti*, observing and counting the number of eggs of Aedes aegypti. Healthy and damaged *Aedes aegypti* on water media contaminated with animal feces and soil.

Method

This study is a descriptive study that describes the egg laying behavior of Ae es. aegypti in water contaminated with animal feces and soil and clean water as control. To find out the egg-laying behavior of *Aedes aegypti* then need to be observed from the introduction of into the cage. After two or three days, the filter paper on all these water media is taken, the number of eggs contained in the filter paper is calculated, the number of healthy and damaged eggs is observed and calculated, and group them according to the type of water and determine the comparison of which water media the eggs are found the most.

The population used in this study was the *Aedes aegypti* females who are full of blood and taken from the Pangandaran Health Research and Development Center. While the sample used was 120 *Aedes aegypti* females who are full of blood and taken from the Pangandaran Health Research and Development Center. Four cages are prepared, each cage is filled with 30 adults full of blood and water media consisting of four kinds, namely: water contaminated with broiler chicken manure, water contaminated with pig manure, water contaminated with soil and clean water as a control.

The data collection technique uses primary data obtained from direct observations of water media contaminated with animal feces and soil. Each animal's manure is mixed with aquades, while the soil is mixed with well water, and four repetitions are carried out with the same type of water medium and the same concentration of 5%. A concentration of 5% is obtained from the calculation results:

5% = 5/100 x 800 = 40 gram

So 40 grams of animal manure and soil are dissolved in 800mL of water.

The instruments used in this study include filter paper, tweezers, beakers, analytical scales, measuring cups, drip pipettes, sample holders, flashlights, cages, Ae es. Adult aegypti, animal feces water, aquadest and clean water.

The research was conducted at the Pangandaran R&D Workshop Laboratory of the Ministry of Health on February 11-14, 2021.

Results

1. Data on the number of eggs on different water media

   The total number of *Aedes aegypti* eggs caught totaled 1,716 eggs, with four repetitions and varying numbers per cage. The results of the *Aedes aegypti* egg-laying behavior test on different water media can be seen in the following table.

TABLE 1 Amount of *Aedes aegypti* Eggs caught inside ovitrap

Media Type	Amount of Eggs Caught on Ovitrap				Amount	Average amount
	1	2	3	4		
Water Contaminated with Broiler Chicken Manure	144	85	144	85	458	115
Water Contaminated with Pig Manure	116	151	110	160	537	134
Soil Contaminated Water	185	216	200	104	705	176
Control (Clean Water)	13	3	0	0	16	4

Based on the results of observations of egg laying behavior in table 1, it shows that the highest number of eggs is found in soil-contaminated water media (705 eggs) while the least number of eggs is found in control media with 16 eggs.

Based on the results of data processing and analysis using the One way Anova test (Table 2), the following results were obtained:

TABLE 2 One Way Anova Test Results

Amount	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	64812,500	3	21604,167	20,096	0,000
Within Groups	12900,500	12	1075,042		
Total	77713,000	15			

Known significant values are 0.000<0.05. This shows that the type of water media contaminated with animal feces and soil used in this study, favored by *Aedes aegypti* as a place to lay eggs.

2. Percentage of *Ae. aegypti* eggs healthy and damaged

   The result of the percentage of eggs Healthy *Aedes aegypti* with damaged water media

contaminated with animal feces and soil, can be seen in the following table:

TABLE 3 Amount of *Aedes aegypti* Eggs Healthy and Damaged

Media Type	Amount of Eggs Caught on Ovitrap								Amount
	1		2		3		4		
	Healthy	Damaged	Healthy	Damaged	Healthy	Damaged	Healthy	Damaged	
Water Contaminated with Broiler Chicken Manure	103	41	70	15	93	51	54	31	458
Water Contaminated with Pig Manure	83	33	111	40	81	29	105	55	537
Soil Contaminated Water	153	32	145	71	134	66	75	29	705
Control (Clean Water)	10	3	2	1	0	0	0	0	16

Table 3 shows the eggs of the *Aedes aegypti* Healthy totaled 1,219 eggs and damaged eggs totaled 497 eggs. Observation of eggs is carried out macroscopically, then the results are processed using the SPSS application with an Independent Sample Test test analysis, the following results are obtained:

TABLE 4 Independent Sample Test Results

Levene's Test or Equality of Variances			t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Jumlah Equal variances								-96,39369	457,39369
Telurassumed Equal variances	1,669	,244	1,595	6	,162	180,50000	113,16047		
not assumed			1,595	3,934	,187	180,50000	113,16047	- 135,7615	496,76152

Table 4 shows that a significant value of 0.162>0.05 indicates no effect of water media on the number of damaged egg percentages. The characteristics of damaged eggs are that they are not oval in shape with a length of ± 0.5 mm, are not smooth, have a lighter color and cannot hatch because the eggs have been damaged.

3. Morphology of healthy eggs produced by *Aedes aegypti*

   Morphology of *Aedes Aegypti* eggs is oval, smooth, ± 0.5 mm long, with stripes on its walls that form a picture resembling gauze webbing. Eggs are white when they are first removed, after 15 minutes the eggs are laid on the medium, the color will change to black. Eggs *Aedes aegypti* can survive for a long time in dry conditions, and will hatch as soon as they are submerged in water. *Aedes Aegypti* eggs after observation can be seen in the picture below:

FIGURE 1 Healthy *Aedes aegypti* eggs observed under a microscope with 40x magnification
(Source : Personal Documentation, February 13, 2021)

Discussion

Based on the results of observations of the laying behavior of *Aedes aegypti* eggs. *Aedes aegypti* released into cages with a temperature of 28.1°C-29.4°C show typical behavior, in general females that have just been released into the cage will rest on the wall before laying eggs (Sabila et al., 2013). However, in this study, they perched on the surface of the water and spread their legs, then dipped their entire bodies until they touched the surface of the water, after which rose again, flew several times and dipped their bodies back into the water. Females lay eggs one by one around the perimeter of moist filter paper.

The results of the study obtained the highest number of eggs found in soil-contaminated water media with a total of 705 eggs. This suggests the soil-contaminated water medium has an attraction for females to lay eggs. In addition, the observations of Lardeux (1992) in Polynesian Tikehau Atoll proved that the *Aedes aegypti* wants to lay her eggs in a medium that is in direct contact with the ground. This evidence is supported by the discovery of *Aedes aegypti* precocious in the well. In Queensland, Australia, 9 of the 10 wells observed reported containing *Aedes aegypti* precocious (Agustina, 2013).

Water media contaminated with pig feces is the second most common water medium for *Aedes aegypti* eggs with a total of 537 eggs. This is because water contaminated with pig manure contains a fairly high amount of ammonia. This ammonia gas is formed due to the process of fecal decomposition by microorganisms that form ammonia gas (NH3), nitrate (NO3), nitrite (NO2) and sulfide gas (H2S). According to (Yossy, 2017), pig manure contains 0.95% nitrogenous nutrients, 0.35% phosphorus, 0.40% potassium, and 60% moisture content. The high nitrogen content in animal feces is influenced by the type of feed given to the animal. The content of these gasses causes a pungent odor and attracts *Aedes aegypti* to lay the eggs in the medium (Kristina & Dewi, 2017).

Water media contaminated with broiler chicken manure became the least number of eggs found compared to water media contaminated with pig manure and soil. Chicken manure contains ammonia which can attract *Aedes aegypti* to come and lay the eggs. Organic waste of chicken manure with special characteristics that have a high nitrogen content of ±2.94% and cause a pungent odor. As it is known that one of the characteristics of chicken manure is its high

ammonia content. Of the three water media used in this study, the ammonia content was quite high, namely from chicken manure, this shows that the *Aedes Aegypti* doesn't really like to make the medium as the laying of her eggs. Christoper (1960) believes that the ammonia content of 120 mg/L can attract females to lay eggs, the ammonia content in culture media exceeding 300 mg/L can be used as a repellent. Therefore, chicken manure produces too high ammonia gas and a pungent odor, which Ae often do not like. aegypti to lay the eggs (Bustan & Pudjirahaju, 2018).

Fresh animal manure contains nitrogen needed for the formation of ammonia. Fresh chicken manure contains 42.18% carbon and ±2.94% nitrogen content which can be a source of ammonia supported by high temperature and humidity. Meanwhile, according to Muliadi (2012) pig manure contains 0.95% nitrogen nutrients and phosphorus nutrients of 0.35% (Bustan & Pudjirahaju, 2018).

The control used in this study was clean water. Clean water is a place used by Ae es. aegypti as a medium of its miss. Theoretically *Aedes Aegypti* breed on clean water and are not in direct contact with groundwater or dirty water. In the control water medium the total eggs found were only 16 eggs. Quite a far different comparison with water media contaminated with animal feces and soil. According to (Agustina, 2013) this is suspected because the water media is contaminated with animal feces and the soil has the availability of organic matter as feed for the survival of its offspring and contains many microorganisms, so the *Aedes Aegypti* is more interested in laying her eggs.

The results of statistical analysis with one way anova showed that the type of media contaminated with animal feces and soil used in the study, had a noticeable influence on the number of eggs laid by with a significant value of 0.000<0.05. Theoretically *Aedes Aegypti* can breed only on clean water, not in direct contact with media contaminated with animal feces or soil. But from the results of this study, it shows that the *Aedes aegypti* has an interest in laying eggs in water media contaminated with animal feces and soil.

The results of a comparative analysis of the number of damaged eggs in each medium showed that the type of water media had no effect on damaged eggs. It can be concluded that the different types of water media used in the study only had a noticeable effect on the number of eggs laid by es, but did not have an influence on the number of damaged eggs.

Based on the results of the study, soil contaminated water (a mixture of between 40 grams of soil dissolved with 800 mL of well water) contains the most number of eggs from the other two media. The results of this study are different from the study conducted by Makna Fathana Sabila in 2013 entitled "Egg Laying Preferences and Inhibition of Precocious Development of Aedes aegypti L In Various Water Media" with groundwater media (30 grams of soil mixture dissolved with 500 mL of well water) showed that the results of laying eggs in groundwater media tended to be lower than those of straw and chlorine soaking water media. In addition, there are differences in the two media used and the concentration of each media is different (Sabila et al., 2013).

The results of observations of egg-laying behavior and the discovery of *Aedes aegypti* eggs on different water media suggests that water contaminated with animal feces and soil can potentially become a place to lay eggs and breed *Aedes aegypti*. Therefore, there is a need for increased vigilance, especially for the community in maintaining a clean and healthy environment.

This study has many limitations including only identifying the egg-laying behavior of

Aedes aegypti and the number of eggs produced from the four media, besides that in this study did not reach the development of the life cycle, where they were observed from adult laying eggs, to larvae, pupae and imago or adult es.

Conclusion

Based on the results of observations of the behavior of *Aedes aegypti* on water media, the contamination of animal feces and soil shows a distinctive behavior, that water contaminated with animal feces and soil is favored by Aedes aegypti as a place for laying eggs and breeding. The total number of eggs caught was 1,716 eggs, with the number of healthy eggs (1,219) eggs, while the number of eggs damaged (497) eggs. The difference in the content contained in the water media affects the laying of *Aedes aegypti* eggs, but does not affect the number of healthy or damaged eggs.

This research can be continued by identifying the development in the life cycle of *Aedes aegypti* with different media and concentrations.

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