# LIFE CYCLE OF *AEDES AEGYPTI* BASED ON ACIDITY VARIATION OF DOMESTIC SEWAGE WATER

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**Abstract.** *Aedes aegypti* breeds in clean water, not in direct contact with the ground, thus, its behavior in breeding becomes the key factor for mosquito survival. Several previous studies showed that *Aedes aegypti* was able to survive and become mosquitoes in environment with high levels of pollution, that were previously considered as unsupportive breeding site, such as domestic sewage water. This study aimed to analyze the difference in *Aedes aegypti's* oviposition, hatchability, and development in various pH of domestic sewage water. This was an experimental study. The number of *Aedes aegypti* gravid females for the oviposition test was 25 per group with five replications with choice and no-choice methods. Eggs produced in the oviposition test were observed for the hatchability and development tests from larvae to imago. Domestic sewage water was maintained at the pH level of 6.5, 7.5, 8.5, and 9.5. Data were analyzed using Kruskal-Wallis and followed by the Mann-Whitney test. There was a significant difference between the oviposition of *Aedes aegypti* in domestic sewage water with various pH used in the choice assay method ($p$ = 0.001) and that in the no-choice assay method ($p$ = 0.003). The eggs of *Aedes aegypti* hatched on Day 9 in sewage water with various pH ($p$ = 0.001), larvae developed into pupae on the seventh day ($p$ = 0.001), and pupae developed into imago on the third day ($p$ = 0.001). Based on this research, we concluded that *Aedes aegypti* can lay eggs not only in clean water but also in domestic sewage water with pH of 6.5-9.5. Eggs hatched in domestic sewage water and developed to be imago at pH 9.5 (18.2%). Thus, it is necessary to socialize the Elimination Breeding Place (EBP) program on domestic sewage water.

**Keywords:** *Aedes aegypti*, domestic sewage water, pH

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## INTRODUCTION

In vector survival, the adaptation behavior of *Aedes aegypti's* in breeding is a key factor, as it is related to the vector-borne disease control program (Mosesa *et al*, 2016). *Aedes aegypti* breeds in clean water that is not in contact with the ground (Mosesa *et al*, 2016), although several studies indicated that the larvae of *Ae. aegypti* can survive to become mosquitoes in polluted water. In Brazil, Chitolina *et al* (2016) showed that the oviposition of *Ae. aegypti* occurs in raw sewage water and their eggs hatch to become larvae and grow into mosquitoes. A research in Kolkata, India by Banerjee *et al* (2015) showed that larvae of *Ae. aegypti* survive and become mosquitoes in environments with high levels of pollution that are previously considered unsupportive breeding places, such as domestic sewage water. Domestic sewage water contains organic materials such as protein, carbohydrates, and lipids and potentially becomes a breeding place for *Ae. aegypti* (Banerjee *et al*, 2015).

Domestic sewage water is water coming from daily human activities related to water use (Triyaswati and Ilmi, 2020). Clean water used for daily households produces domestic sewage water containing alkaline compounds, such as NaOH and KOH with pH of 6-9, coming from bath soap or detergent used for daily life (Martini *et al*, 2019). According to Wuwungan *et al* (2013), *Ae. aegypti* larvae may become mosquitoes in soapy water media with a pH of 7-8. Similarly, research by Burke *et al* (2010) in Playa-Playita, Puerto Rico, concluded that *Ae. aegypti* can get into the cracks of the septic tank to lay eggs and to become imagoes in septic tank sewage water with a pH of 7.56. Meanwhile, a study by Martini *et al* (2019) in Semarang, Central Java, Central Java, showed that *Ae. aegypti* survives and potentially becomes a dengue hemorrhagic fever vector in domestic sewage waste with a pH of 8.7.

A preliminary study conducted in the middle of September 2021 by analyzing 25 samples of domestic sewage water at Gilimanuk sub-district, Jembrana Regency, Bali, showed that the average pH of domestic sewage water was 9. The characteristic of alkaline sewage water was that people directly dispose of waste containing soap from the bathroom and kitchen. Further observation of *Ae. aegypti* oviposition showed that mosquitoes could lay eggs in ovitraps containing liquid waste with a pH of 6.5 to 9.5. The eggs produced were able to hatch and grew into imago with pH values of 6.5 and 9.5. According to Delfita (2018), sewers are flooded and become transparent, potentially creating breeding places for *Ae. aegypti*, so people need to be aware of sewer water as a breeding place for *Ae. aegypti*.

## MATERIALS AND METHODS

The equipment used in this research was a mosquito cage of 30 x 30 x 30 cm, ovitraps, pH meter (PH-009, ATC, South Tanggerang, Indonesia), air thermometer (GEA Medical, North Jakarta, Indonesia), water thermometer (SK SATO, Tokyo, Japan), turbidimeter (Turbiquant® 1100 Portable Turbidity Meters, Merck, Berlin, Germany), domestic sewage water collected from Gilimanuk subdistrict (Jembrana-Bali), NaOH 0.1 M (Merck, East Jakarta, Indonesia), HCl 0.1 M (Merck, East Jakarta, Indonesia), Aquadest (Smartlab, South Tanggerang, Indonesia), and female *Ae. aegypti* gravid I (fed with guinea pig blood for two hours for egg maturation).

This research was an experimental study. Replications using the Federer formula (t - 1) (r - 1) ≥15 and five replications were determined. The research was conducted in the Entomology Laboratory of Health Quarantine Office Class I Denpasar. The total samples for each group, 25 females and males *Ae.* aged 4-5 days, were put into cages of 30 x 30 x 30 cm for one day to allow the copulation. The females were separated from the male mosquito and fed with guinea pigs' blood for two hours. After the mosquitoes were confirmed to become female *Ae. aegypti* gravid I, they were reared in cages without ovitrap for 5 days to ensure that the mosquitoes could lay eggs during the oviposition test. During the research, the laboratory conditions were maintained at a temperature of 25-30℃ and air humidity of 81.5-89.5%. (Anggraini and Cahyati, 2017).

Domestic sewage water samples were taken from drainage around the Gilimanuk sub-district, Bali, Indonesia. The temperature of the domestic sewage water during the study was maintained at intervals of temperature (24.1±2℃) and turbidity (31.14±16.3 NTU) so that the sewage water sample had the same characteristics. The sewage water sample was divided into four containers and maintained by adding 0.1 M NaOH or 0.1 M HCl to obtain sewage water with a pH of 6.5, pH of 7.5, pH of 8.5, and pH of 9.5. Domestic sewage water was used for the treatment group.

The females *Ae. aegypti* gravid I were kept in cages without access to an ovitrap for five days. On the fifth day, the ovitraps were placed in the oviposition cage. The Oviposition used the choice assay and no-choice assay methods. Each method consisted of a treatment group with pH 6.5, pH 7.5, 8.5, and 9.5, positive control (aquadest), and a negative control (1% sodium hypochlorite solution). In the choice assay method, the ovitraps with various pH levels of domestic sewage water and positive and negative controls were placed in one cage. The oviposition of the choice assay method was also supplemented with psychological control to ensure that the *Aedes aegypti* gravid I, female, was in a condition ready to lay eggs. In the no-choice assay method, each cage was only filled with one pH concentration. The ovitrap and domestic sewage water were replaced daily with the same waste treatment, and the ovitrap position was rotated clockwise daily. Observations were made for three days (72 hours) by counting eggs daily. The oviposition design is shown in Fig 1.

Eggs from the oviposition test in the treatment and positive control groups were continued with the hatchability test. Eggs were placed in a tray filled with 250 ml of domestic liquid waste with pH of 6.5, 7.5, 8.5, and 9.5, and one control with pH 7.3 containing aquadest. The eggs were hatched until the appearance of the last *Ae. aegypti* larvae of the first instar.

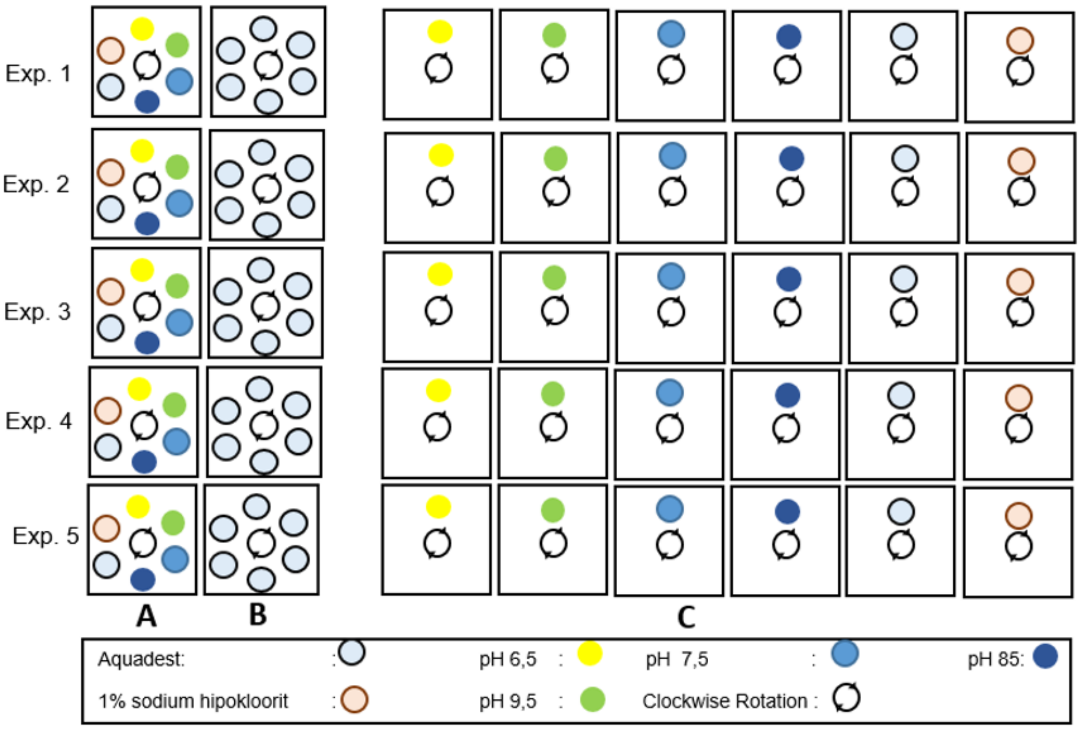


Fig 1 - Ovipositional study design

A: Experimental cage in the choice assay method; B: Psychological control cage in the choice assay method; C: Experimental cage in the non-choice assay method

The controls were fed with dog food 2 gr/day since the appearance of the first larvae. The test was repeated 5 times. Larvae were observed until the appearance of the last pupae. The pupae were transferred to a container containing sewage. The number of imagoes was observed every day until the appearance of the last imago. During the test, hatching data and the development of temperature and turbidity of the liquid waste were recorded every 8 hours. The data were presented in percentage and analyzed using the Kruskal Wallis test and followed by Mann Whitney test to analyze the difference in *Aedes aegypti*'s oviposition, hatchability, and development in various pH of domestic sewage water.

## RESULTS

The oviposition test was carried out by two methods: choice assay and no-choice assay. During the oviposition test, the average air temperature was 26.2°C, and the average humidity was 82.4%. The test showed similar oviposition results in both methods. The more alkaline the domestic sewage water, the lower the average number of eggs produced. The result of the oviposition test is shown in Table 1:

The result of the oviposition test with the choice assay method showed the average number of *Ae. aegypti* eggs in positive control and psychological

Table 1
*Aedes aegypti* oviposition test

| Method | Average number of Aedes aegypti egg | | | | | | |
|---|---|---|---|---|---|---|---|
| | pH 6.5 | pH 7.5 | pH 8.5 | pH 9.5 | Positive | Negative | With psychological control |
| Choice | 225.2[b] | 250.8[b] | 131.4[a] | 96.2[a] | 258.8[b] | 0 | 281.3 |
| No-choice | 733.6[b] | 793.8[a] | 646.2[b] | 531.6[b] | 1.191[b] | 0 | - |

[a]significantly different at $p<0.05$ by the Mann-Whitney U test; [b]$p \geq 0.05$ by the Mann-Whitney U test

Note: The oviposition used the choice assay and no-choice assay methods. Each method consisted of a treatment group with pH 6.5, pH 7.5, 8.5, and 9.5, positive control (aquadest), and a negative control (1% sodium hypochlorite solution). In the choice assay method, the ovitraps with various pH levels of domestic sewage water and positive and negative controls were placed in one cage. In the choice assay method, the ovitraps with various pH levels of domestic sewage water and positive and negative controls were placed in one cage. The oviposition of the choice assay method was also supplemented with psychological control to ensure that the Aedes aegypti gravid I, female, was in a condition ready to lay eggs. In the no-choice assay method, each cage was only filled with one pH concentration.

control had a similar number, indicating that the sample of female *Ae. aegypti* was in good condition at the time of the oviposition test. The lowest egg average was found at pH 9.5, which was 96.2 eggs. A similar result was found in the oviposition test with the non-choice assay method. The lowest average eggs were found at pH 9.5, which was 531.6. The results of the Kruskal-Wallis test showed a significant difference between the acidity variations of domestic sewage water to the oviposition of *Ae. aegypti* in the choice assay method ($p = 0.001$) and the no-choice assay method ($p = 0.003$). The Mann–Whitney test analyzed differences in oviposition in each group according to the notation in Table 1. The notation indicated a significant difference between the average eggs produced by the choice assay method oviposition at the pH of domestic sewage water at pH 8.5 and pH 9.5 with others. Meanwhile, in the no-choice assay method, a significant difference was shown in the average number of eggs produced in domestic sewage water at pH 8.5 compared with the others.

The oviposition test was continued to observe the hatchability and larval development until the imago stage. The hatchability test sample used the eggs produced from the oviposition test. Eggs were hatched in domestic sewage water with pH 6.5, 7.5, 8.5, and 9.5, and the control was only filled with aquadest. The average temperature of the domestic sewage water during the study was in the range of 25.6-26.0°C, and the turbidity in control was 2 NTU, while the domestic sewage water was in the range of 34.7-34.9 NTU. The results of hatchability and development tests are shown in Table 2.

The highest average number of *Ae. aegypti* egg hatched into larvae in the control group was 1358 (93.67%), while the average number of *Ae. aegypti* was in the treatment group egg hatched into larvae was 449.4 (40.68%) at pH of 7.5 and the lowest was 31.2 (4.97%) at pH of 9.5. Egg hatchability was observed daily to determine the hatchability of eggs daily. The results of observations of hatchability per day are shown in Fig 2. The eggs hatched into larvae and continued to grow every day. Larvae of *Ae. aegypti* appeared in domestic sewage water from the first day until the ninth day. The highest percentage of eggs hatched was in the control group at 93.67%, and the lowest was at a pH of 9.5 which was 5%. The Kruskal-Wallis test showed a

Table 2

Hatchability and development of *Aedes aegypti* larvae to imago in various acidity of domestic sewage water

| Sewage acidity | Average number of mosquito larvae count, mean ± SD (% Hatchability of eggs) | Average number of pupae count, mean ± SD (% Development from larvae to pupae) | Average number of imago count, mean ± SD (% Development from pupae to imago) |
|---|---|---|---|
| Control (pH 7.3) | 1,358 ± 151.1[a] (93.67) | 1,322.6 ± 142.1[a] (97.4) | 1278 ± 62.3[a] (96.6) |
| pH 6.5 | 202.6 ± 22.7[a] (21.13) | 91.2 ± 4.3[a] (44.8) | 22.8 ± 1.8[a] (25.0) |
| pH 7.5 | 449.4 ± 66.0[a] (40.68) | 311.6 ± 10.3[b] (68.9) | 198.8 ± 14.5[a] (63.8) |
| pH 8.5 | 101.4 ± 9.0[a] (13.04) | 53.4 ± 7.5[a] (41.3) | 14.6 ± 2.8[a] (34.8) |
| pH 9.5 | 31.2 ± 2.5[b] (4.97) | 2.2 ± 0.3[a] (7.1) | 0.4 ± 0.05[a] (18.2) |

[a]significantly different at $p<0.05$ by the Mann-Whitney U test; [b]$p \geq 0.05$ by the Mann-Whitney U test

SD: standard deviation

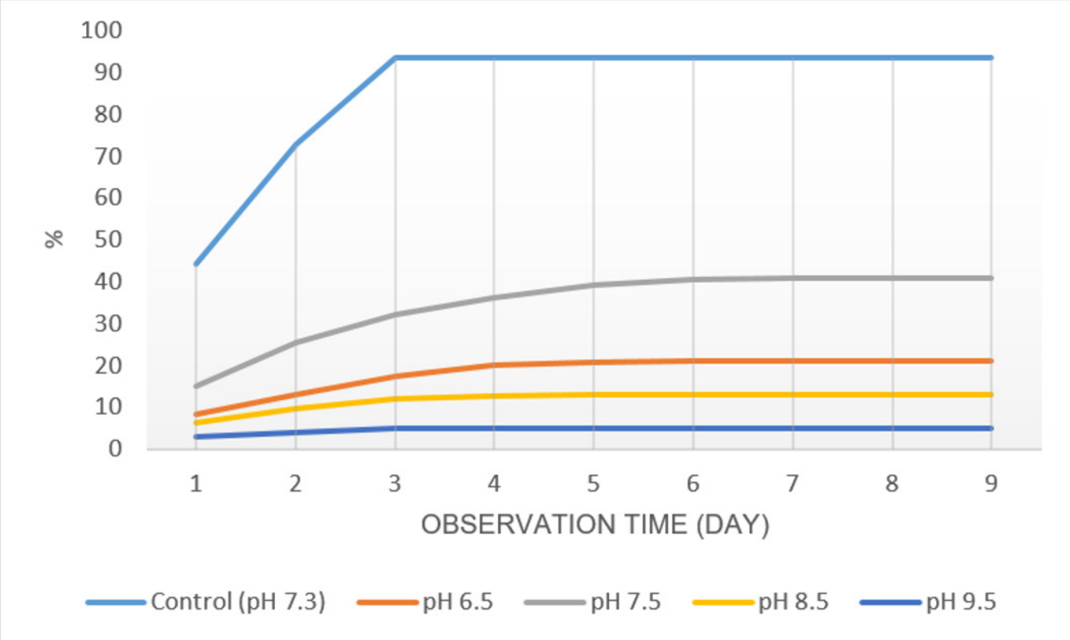


Fig 2 - Percentage of hatchability of *Aedes aegypti* eggs in various acidities of domestic sewage water

significant difference in the pH variation of domestic sewage water on eggs' hatchability of *Ae. aegypti* ($p$ = 0.001). The results of the Mann-Whitney U test showed a significant difference between the number of eggs produced at the pH of domestic sewage water at pH of 9.5 and the others.

*Ae. aegypti* larvae from the hatchability test were observed on each tray filled with 250 ml of domestic liquid waste with a pH of 6.5, 7.5, 8.5, and 9.5, and control containing aquadest with an average pH of 7.3. The test results showed the highest average larvae of *Ae. aegypti* that developed into pupae were in the control group which was 91.2%, while in the treatment group, the average *Ae. aegypti* larvae that developed into pupae were at pH 7.5 (28.2%) and the lowest at pH 9.5 (0.4%). The development of *Ae. aegypti* larvae into pupae was observed every day to determine the number of pupae that appeared per day. The results of observations of larval development per day are shown in Fig 3. The number of larvae that develop into pupae in various domestic sewage water started from the fifth day, and the last pupae appearred on the seventh day. On the seventh day, domestic sewage water with a pH of 9.5 was the lowest to contain pupae at 0.4%. The results of the Kruskal-Wallis test showed a significant difference in the pH variation of domestic sewage water on the development of *Ae. aegypti* to pupa stage ($p = 0.001$). Furthermore, the analysis was continued with the Mann-Whitney U test with the result that there was a significant difference between the number of pupae produced at the pH of domestic sewage water at pH of 6.5, 8.5, and 9.5.

The *Ae. aegypti* pupae were observed for the development in sewage water with various acidity until the appearance of the last imago (adult mosquitoes). The results of the observations showed that the highest number

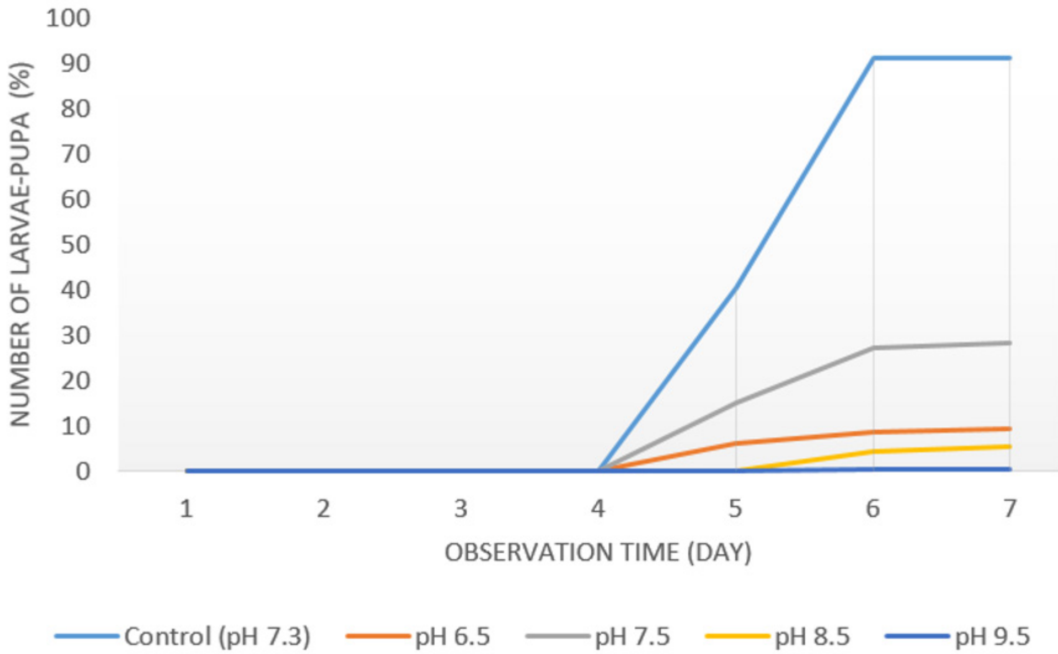


Fig 3 - Percentage of larva-pupa development of *Aedes aegypti* per day of *Ae. aegypti* pupae developed into imago were 1,278 (96.6%) in the control group. In the treatment group, the highest number of *Aedes aegypti* pupa developed into imago was 198.8 (18%) at pH 7.5, and the lowest was 0.4 (0.1%) at pH 9.5. The development of *Ae. aegypti* pupae into imago were observed daily, according to Fig 4. The number of pupae developed into imago in various domestic sewage water was from the seventh day until the ninth day. On the ninth day, domestic sewage water with pH 9.5 was the lowest imago (0.1%). The results of the Kruskal-Wallis test showed a significant difference in domestic sewage water with various acidities to the development of *Ae. aegypti* pupa into imago stage ($p = 0.001$). Furthermore, the Mann-Whitney U test was carried out with the results showing a significant difference between the numbers of imagoes produced at the pH of domestic sewage water at a pH of 6.5, 7.5, 8.5, and 9.5.

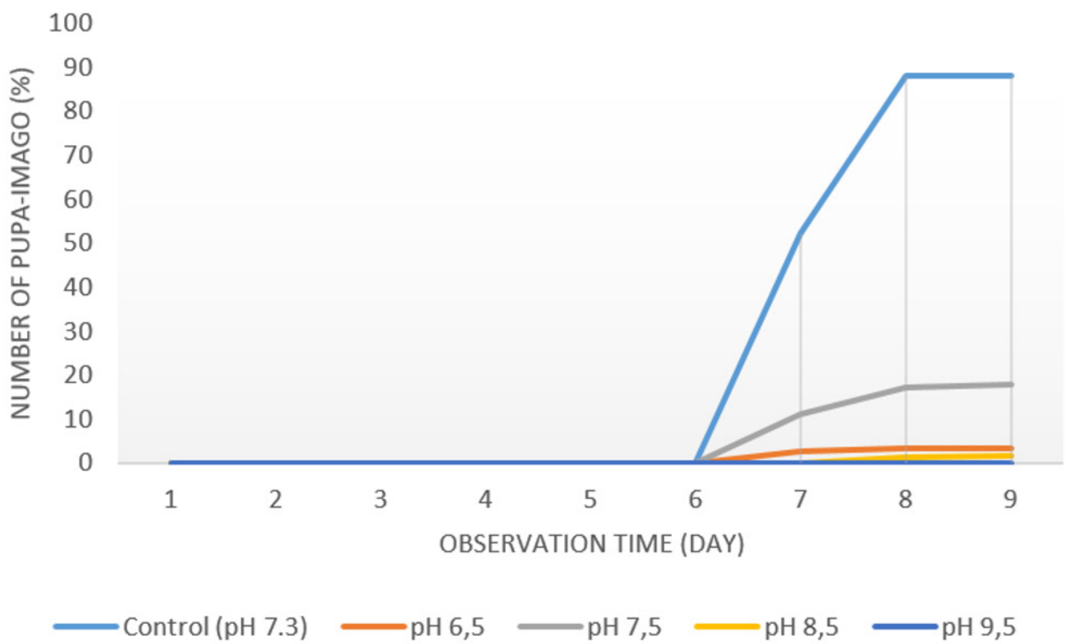


Fig 4 - Percentage of pupa-imago development of *Aedes aegypti* per day

## DISCUSSION

This research showed that, on a laboratory scale, *Ae. aegypti* performed oviposition and development in the pH range of 6.5 to 9.5. The average number of eggs produced in the oviposition choice assay method and the no-choice oviposition method showed a similar result. The highest average number of eggs was found in the positive control, which only contained aquadest, while in the treatment group, the highest average was found at pH 7,5, and the lowest was at pH 9.5. The results of this research suggested that *Ae. aegypti* was able to lay the egg in both clean water and domestic sewage water media with various pH concentrations. A study in Puerto Rico showed that *Ae. aegypti* can enter the cracks in the septic tank and lay the eggs into liquid waste with a pH of 7.56 (Burke *et al*, 2010). Meanwhile, a study in India showed that larvae of *Ae. aegypti* lay their eggs in liquid waste due to changes in the adaptation behavior of *Ae. aegypti* (Banerjee *et al*, 2015). Based on this research, it is necessary to consider sewage water as a breeding place for *Ae. aegypti*.

Statistical analysis demonstrated a significant difference in the variation acidity of domestic sewage water to the oviposition of *Ae. aegypti* in the choice assay method ($p = 0.001$) and no-choice assay ($p = 0.003$). The results of the Mann-Whitney test for each group showed a significant difference in the oviposition of the choice assay method at pH 8.5 and pH 9.5. In contrast, in the no-choice assay method, a significant difference occurred at pH 7.5. It showed that the more alkaline the domestic sewage water concentration, the lower the average number of eggs in the oviposition test. *Ae. aegypti* gravid I females were kept in cages without ovitrap access for five days. This procedure aimed to increase the need for mosquitoes to ovipose and increase mosquito preferences in choosing egg oviposition media (Chitolina *et al*, 2016). In nature, the mosquito *Ae. aegypti* chooses the best place to lay the egg on different media; this behavior is called skip oviposition (Abreu *et al*, 2015). The oviposition test indicated that domestic sewage water with a pH range of 6.5 to 9.5 did not prevent *Ae. aegypti* from oviposition.

The variation of acidity affected the quality of the chemical parameters of sewage water and supported mosquitoes in laying eggs. In theory, domestic sewage water with a low pH concentration produces ammonia compounds (Martini *et al*, 2019). Ammonia in domestic sewage water comes from the oxidation of organic substances both chemically or due to the role of microorganisms at low pH (Putri *et al*, 2019). The higher of pH concentration, the more ammonia is lost. Increasing the pH of the solution will increase the concentration of OH- ions, thereby providing alkaline conditions for ammonia oxidation (Retnoningsih and Murdianti, 2010). Therefore, at a more alkaline pH, the lower number of *Ae. aegypti* eggs were found. The smell of ammonia affects the oviposition of *Ae. aegypti*, which chooses the medium for laying eggs (Martini *et al*, 2019). The ammonia attracts the olfactory organs, or sense of smell, of female *Ae. aegypti*. Higher ammonia levels in the sewage water will increase the chances of mosquitos' ovipose (Anggraini and Cahyati, 2017). Therefore, it is necessary to know that domestic sewage can be a breeding place for *Ae. aegypti*.

The eggs produced from the oviposition test were continued with the hatchability test. The hatchability test's average media temperature was 25.6-26.0°C. This condition is the optimum condition for hatching eggs at a temperature of 23-27°C (Yulidar, 2014). The results of this study showed that the average eggs hatched into first instar larvae occurred in the control group (93.67%), while in waste, the average number of eggs hatched the most at pH 7.5 (40.68%) and the lowest at pH 9.5 (4.97%). This study confirmed the eggs of *Ae. aegypti* hatched in clean water and domestic sewage water with various pH. The results of this study were in line with the research of Mataram and Warni (2017) that the eggs of *Ae. aegypti* was able to hatch in sewage water with pH 8.3 and other media such as tidal water, rainwater, and groundwater.

During the research, the average turbidity of the liquid waste media was 34.7-34.9 NTU. This indicated that the sewage water had a high level of turbidity. The turbidity of the sewage water affected the development of *Ae. aegypti*. Turbidity describes the optical properties that cause the phenomenon of light refraction and the obstruction of sunlight penetration into the water. Turbidity is caused by the entry of substances that are not suspended or suspended in water. Excessive levels of turbidity affect the changes in insect body structure, such as abrasion of the respiratory tract epithelium, decreased feeding frequency, blocked breathing system, exposure to toxic materials, and reduced vision, while the impact of turbidity also reduces dissolved oxygen (Mataram and Warni, 2017).

The results of the Kruskal-Wallis analysis indicated a significant difference in various acidity of domestic sewage water to the development of *Ae. aegypti* to the pupa stage. This study showed that the larvae of *Ae. aegypti* was able to develop into pupae either on control media containing aquadest or sewage waste with various acidity. The Man-Whitney test results showed a significant difference between pH 6.5, 8.5, and 9.5. The more alkaline the development of the larval medium, the less the number of pupae will appear.

The alkaline nature of sewage water is due to detergent compounds. Detergents will produce surfactant compounds such as organic substances that easily bind oxygen, interfering with the larval process of taking oxygen. This condition will increase larval mortality and prevent larvae from developing into pupae (Martini *et al*, 2017). In this study, the development of larvae into pupae was still found in acidic conditions (pH 6.5), the average number of pupae that appeared was 44.8%. It was because pH 6.5 is classified as a weak acid, allowing the larvae to develop into pupae. According to Janah and Pawenang (2017), larvae of *Ae. aegypti* could not develop into pupae at a pH level of 3, which is strongly acidic. In this study, the larvae developed into pupae on the fifth to the seventh day. At the most alkaline pH of the sewage water, the ability of larvae to develop into pupae only reached 7.1%. It indicates that the pH of the developmental medium, which is acidic and alkaline, also affects the insect ecdysone hormone system. Ecdysone hormone plays a role in the development of adult characteristics of insects, including the development of larvae into pupae (Hadi *et al*, 2011).

Pupae observations were conducted until the presence of the last imago. The lowest number of imago was at pH 9.5, 18.2%. Pupae develop into imago on waste media, which takes three days. This was different from the pupa development of *Ae. aegypti* in nature, which takes an average of two days (Ridha *et al*, 2017). It indicates that contaminated media decreases the development of pupae in imago. The content of household sewage water that contains a high level of soap substances will cause the pH of the waste to become alkaline and contains detergent compounds. The content of household sewage water with a high pH causes the development of larvae into pupae and pupae into imago (Martini *et al*, 2019).

This study proved that the *Ae. aegypti* grew and developed in clean and domestic sewage water with a variation of acidity. This result was in line with Jacob *et al* (2014) which showed that *Ae. aegypti* survive and usually develop from larvae to imago stage in sewage water. According to Wurisastuti (2013), the ability of *Ae. aegypti* to develop until imago in sewage water indicate changes in the behavior of *Ae. aegypti* in choosing a breeding place and the ability to develop in a polluted environment. If mosquitoes do not find a breeding place in clean water, then *Ae. aegypti* switch to other polluted places that contain factors that support the life cycle.

In summary, female *Ae. aegypti* mosquitos can adapt to polluted breeding sites. The results of laboratory-scale research showed that *Ae. aegypti* can lay eggs not only in clean water but also in domestic sewage water with a pH of 6.5-9.5. Eggs hatch in domestic sewage water and developed to be imago at pH 9.5 (18.2%). These findings can be considered in future vector control measures. In addition, to strengthen the results of this study, it is necessary to conduct further research on a field scale. Applying larvicides to containers where *Ae. aegypti* larvae are found so that the larvae do not move into the drainage when draining water containers and develop into *Ae. aegypti* mosquito vectors in sewage water. Monitoring the presence of *Ae. aegypti* larvae not only in clean water but also domestic sewage water with alkaline pH.

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## CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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