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Submission date: 15-May-2020 09:13AM (UTC+0700)
Submission ID: 1324613818
File name: -_Artikel_Longevity_and_development_of_Aedes_aegypti_larvae.pdf (306.3K)
Word count: 3776
Character count: 18920

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Article · January 2019
DOI: 10.19106/JMedSci005104201906

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Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

JMedSci

Volume 51, Number 4, 2019; 325-332
http://dx.doi.org/10.19106/JMedSci005104201906

Longevity and development of *Aedes aegypti* larvae to imago in domestic sewage water

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Submited: 2018-01-22
Accepted : 2019-09-16

ABSTRACT

Dengue Hemorrhagic Fever (DHF) caused by dengue virus transmitted by *Aedes aegypti* (*Ae. Aegypti*) that lives and breeds in clean water. The aim of the study was to analyze the difference of the longevity and development of *Ae. aegypti* larva in the difference pH of domestic sewage water. This experimental used post only control group design to analyze population of *Ae. aegypti* instar III larvae which was bred in the Entomology Laboratory of the Faculty of Public Health, Diponegoro University, Semarang, Central Java. The number of tested larvae was 25 per media with six replications. Thus, the total number of larvae was 750. Data were analyzed using Kruskal Wallis and followed by Mann Whitney test. The result showed that *Ae. aegypti* larva could survive and breed to be mosquitos in the domestic sewage water with various pH levels. There was significant difference between the number of larvae transforming to pupa ($p=0.002$), pupa to imago ($p=0.001$), and the number of survival imago until second week ($p<0.001$) in the domestic waste water with various pH levels. Other findings revealed that people tended to wash away larvae they found in the water, but still used the water for daily live. As a result, the larvae bred in the domestic sewage water. Therefore, elimination breeding place (EBP) program needs to be socialized to make people aware of either domestic waste water or domestic clean water.

ABSTRAK

Demam berdarah dengue (DBD) yang disebabkan oleh virus dengue ditularkan oleh *Aedes aegypti* (*Ae. aegypti*) yang hidup dan berkembangbiak di air bersih. Tujuan penelitian ini adalah untuk menganalisis perbedaan umur panjang dan perkembangan larva *Ae. aegypti* dalam perbedaan pH air limbah domestik. Penelitian ini menggunakan desain kelompok kontrol *post-only* untuk menganalisis populasi larva *Ae. aegypti* instar III, berkembangbiak di Laboratorium Entomologi, Fakultas Kesehatan Masyarakat, Universitas Diponegoro, Semarang, Jawa Tengah. Jumlah uji larva adalah 25/media dengan enam ulangan. Dengan demikian, jumlah total larva adalah 750. Data dianalisis menggunakan Kruskal Wallis dan diikuti dengan uji Mann Whitney. Hasil penelitian menunjukkan bahwa larva *Ae. aegypti* dapat bertahan hidup dan berkembangbiak menjadi nyamuk di air limbah domestik dengan berbagai tingkat pH. Ada perbedaan yang signifikan antara jumlah larva yang berubah menjadi pupa ($p = 0{,}002$), pupa menjadi imago ($p = 0{,}001$), dan jumlah imago yang bertahan hingga minggu kedua ($p < 0{,}001$) di air limbah domestik dengan berbagai tingkat pH. Temuan lain mengungkap bahwa orang cenderung membuang larva yang ditemukan di dalam air, tetapi masih menggunakan air untuk kehidupan sehari-hari. Akibatnya, larva berkembang biak di air limbah domestik. Oleh karena itu, program pemusnahan tempat perindukan nyamuk perlu disosialisasikan agar masyarakat sadar akan air limbah domestik atau air bersih domestik.

Keywords:
Ae. aegypti
larvae
breeding places
DHF
EBP

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by dengue virus transmitted by *Aedes* sp mosquito. Dengue fever, a fatal disease spread out in both tropical and sub-tropical zones, widely infects people in many countries in South East Asia, including Indonesia.[1] The vector of DHF disease in Indonesia is *Aedes* sp, particularly *Ae. Aegypti*. However, *Ae. albopictus* can also be the vector.[2] The dengue virus is an RNA virus that is part of the Flaviviridae family and comes from the genus Flavivirus. This virus has four serotypes known as DEN-1, DEN-2, DEN-3 and DEN-4. During this clinic it has different manifestations depending on the dengue virus serotype.[3] *Aedes aegypti* usually breeds in clean water reservoir.[4]

In preventing the spread of DHF vector, the government has implemented a program called elimination breeding place (EBP) by doing DCR plus (draining, closing, recycling) plus other activities related to mosquitoes breeding place elimination.[5] The EBP is a government introduced program and highly recommended to prevent and control the spread of DHF vector.

However, the impact of the implementation of the EBP has still been assessing, especially the one related to the steps in conducting DCR. Among the three steps, draining container has become the focus of the study. Draining water containing mosquito larva without filtering and taking the larva out provides opportunity for the larvae to be adult, as *Ae. aegypti* larvae survive in domestic waste water.[6]

Clean water used for the daily needs produces domestic liquid waste. The wasted water from bathroom containing soap (containing NaOH and KOH/alkali) is the one most produces by either city or rural people.[7] The domestic waste water has an approximate pH at 6-9 according to the standard quality of domestic waste. Laboratory tests showed that the development of *Ae. aegypti* eggs occur more quickly in soapy water than clear water.[8] Some research results also showed that *Ae. aegypti* eggs are found in an ovitrap filled with straw soaking water.[9]

Preliminary study conducted in Tembalang sub-district, Semarang City, Central Java by analyzing 20 samples of domestic wasted water showed that the average pH of wasted water was 8.5. Once an *Ae. aegypti* larvae survives in the water reservoir, it will be growing to be an adult (imago stadium). Mardihusodo[8] showed that bath soap and pam waste water is the most chosen and optimal place in the development of *Ae. aegypti* larvae becomes imago. However, no research on the durability and long life cycle of larvae to become an imago or even a mosquitoes in household waste water was conducted. In contrast, the endurance of *Ae. aegypti* larvae in polluted water without looking at growth and the length of its life cycle was reported. Moreover, a larval endurance tests on *Ae. aegypti* in soapy water reported all the larvae die due to the high alkaline of water with pH of 12.8. It is known that the pH of waste water not high where mosquito larvae can well survive. It is reported mosquito larvae can not develop at pH <3 or >12.[6]

MATERIALS AND METHODS

Materials

The subject in this research was *Ae. aegypti* on third stadium of larva or instar III. They were chosen as mosquitoe's organs have been fully formed, and relatively stable from the outside effect. The instar III larvae of *Ae. aegypti* were obtained from the Laboratory of Faculty of Public Health, Diponegoro University, Semarang, Center of Java. The number of larvae were 25 as suggested by WHO in conducting bioassay test. The larvae tested were placed in aplastic jar while their growth, development, and until die observed.

The domestic waste samples were taken from liquid waste samples from drainage ditch around houses in Tembalang Sub-district, Semarang in mid of May 2017. The Tembalang Sub-district was chosen in respond to information delivered by health cadres that most people in village still used bath, while people in other villages had used bucket or shower. They take a bath using bucket or shower minimize where the possibility of *Ae. aegypti* eggs become larvae because the water would be disposed after being used for households' activities.

Methods

An experimental research with post only control group design was implemented by splitting the experiment into two groups i.e. experimental and control groups. Federer formula was used to replicate the experiment. The formula is (t-1) (r-1) ≥ 15, where t was number of intervention (n = 5), and r was number of replication.

This study was conducted in the Entomology Laboratory, Faculty of Public Health, Diponegoro University, Semarang, Central Java where the population of *Ae. aegypti* larvae were reared. The pH level of waste sample applied was five (including control) i.e. 7.5 – 8, 8.1 – 8.5, 8.6 – 9, and 9.1 – 9.5. Each level of concentration was replicate six times. The level of pH was determined by the pH of waste water samples taken from Tembalang Sub district.

The research procedure was conducted as follow a) 250 mL waste water samples were collected from Tembalang Sub-district and divided it into pH interval group i.e. 7.5 – 8, 8.1 – 8.5, 8.6 – 9, and 9.1 – 9.5. b) the waste water samples were then placed in the plastic jars, in which 25 instar III larvae of *Ae. aegypti* were put in every plastic jar. c) the development from the stage of larvae to be adult and die was observed. d) during the cycle of their live, the instar III larvae were fed following their development stages. E) the observation was conducted during 4 weeks.

Data analysis

The data were presented in percentage and analyzed using the Kruskal Wallis test and followed by Mann Whitney test, because the data were not normally distributed. A p value <0.05 was considered significant.

RESULTS

The number of larva reared into pupas increased every day and the highest one was observed in control group. Meanwhile, the lowest of the total larvae reared into pupas was found in group with the highest pH (9.3). The instar III larvae started to be pupas in the third day (FIGURE 1). Further, the pupas started to be imago took place in the 5th day with the peak was in the 6th day, and the lowest number was found in the group with the highest pH level of 9.3 (FIGURE 2).

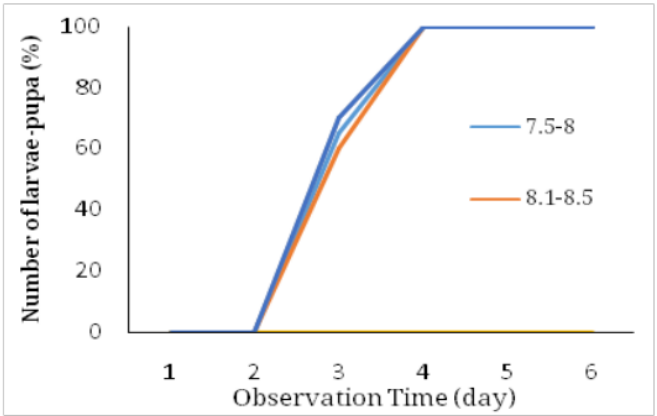

FIGURE 1. The number of larvae (%) reared into pupas

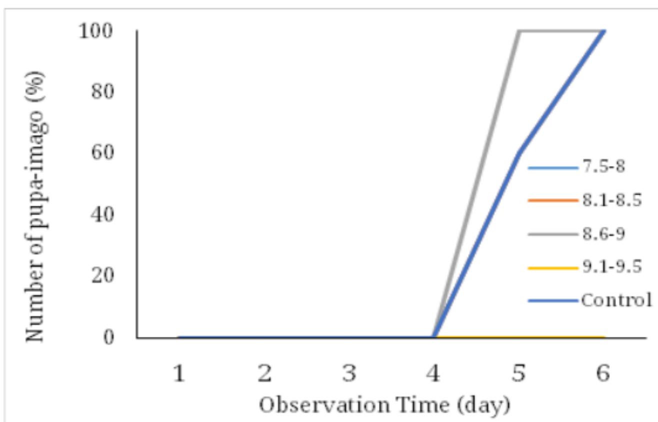

FIGURE 2. The number of pupas (%) reared into imago

The survival pupa from week to week is presented in FIGURE 3. On the second week, the highest number of imago died was found in the group belonged to the domestic wasted water with pH of 8.7. The percentage of living imago gradually decreased during the observation and in the 4th week, only few imago survived with the highest number was found in the control group.

During the study, the temperature and humidly in the entomology laboratory was measured. The average temperature was 26.2˚C, and the average relative humidity was 79%. The temperature of the sewage water media to rear the *Aedes* sp was 24-27˚C, while the variables of the environment did not show extreme fluctuation.

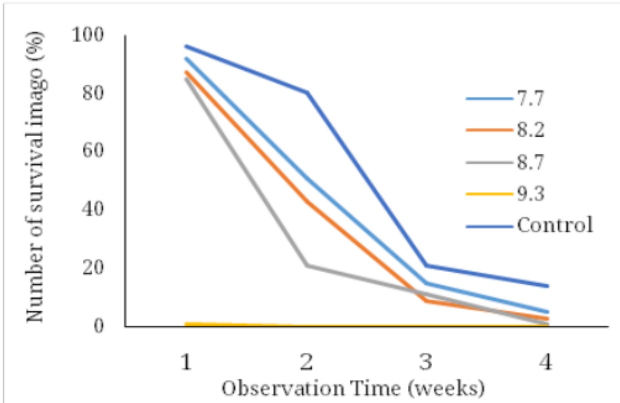

FIGURE 3. The longevity of *Ae. aegypti* per week

As the data were not normally distributed (p=0.006), they were analyzed with Kruskal-Wallis test and Mann-Whitney U test and the statistic test result showed that p=0.002. Therefore, there was a significant difference between the number of larvae reared into pupas in several pH of domestic wasted water; the number of *Ae. aegypti* pupas grew into imago (p=0.001); and the number of imago survived until 2 weeks in several pH of the domestic wasted water (p<0.0001), as described in TABLE 1. The highest pH on 9.3 commonly showed significance among the pH level and the control group. The larvae to pupas could not develop well inn pH more than 9.0.

TABLE 1. The longevity variance from larva to imago in the domestic wasted water in various of pH level

pH of wasted water	Larva to pupa	%	Pupa to imago	%	Longevity of imago	%
Control	24.5 [a]	98.0	4.0 [a]	96.0	20.0 [d]	96.0
7.7	24.2 [a]	96.7	23.0 [a]	92.0	13.2 [e]	92.0
8.2	22.7 [a]	90.7	21.8 [a]	87.3	11.0 [c]	87.3
8.7	22.3 [a]	89.3	21.2 [a]	84.7	5.0 [b]	84.7
9.3	1.3 [b]	5.3	0.2 [b]	0.7	0.0 [a]	0.7

Note: The notation of small letter accompanying a number was significantly different in p = 0.05 by Mann-Whitney U test

DISCUSSION

The average number of dead larvae in the pH 9.3 of the wasted water was 18 in every replication. It was suspected that as the media belonged to the most alkali one, containing high concentration of detergent, the larvae could not survive. Detergent has potential as larvicide for the *Ae. aegypti* larvae. The ionic cluster has strong affinity to water, while the hydrocarbon cluster has not.[10] It was reported that high concentration of detergent produces high concentration of surfactant in the media of *Ae.aegypti* larvae. Surfactant is active organic substance that can interfere the oxygen taken by larvae leading to their dead.[11]

The pH of the media changed from the initial condition to gradually become acidulous caused by microorganism activities. At the same time, the microorganism might become additional feed for larvae. Sudarmaja dan Mardihusodo[12] proved that waste water containing soap and tap water are the most optimum media for *Ae. aegypti* larva to grow to become imagos. Patterson[13] reported that household waste containing detergents and other chemical substances can greatly influence water quality such as pH and ammonia levels. Ammonia has been implicated as one of the primary toxic compounds limiting larvae of *Ae. aegypti*.

Further analysis showed that there was no significant difference among larva growing in the domestic waste water with the initial pH of 7.7, 8.2, and 8.7. This finding showed that the *Ae. aegypti* larva could also rear into pupa in the household waste water environment with normal pH. Moreover, the domestic waste water containing chemicals was normal and fit for the larva to live. Other possibility was that the turbidity of the waste water gradually decreased, as its particles precipitated in the bottom of the jar, and; therefore, the larvae grew into imago as if they grew in the clean water.[14]

The fact that *Ae. aegypti* larva were able to grow into imago not in the normal pH water of 6.5-7.5. It suggested that the *Ae. aegypti* larva could adapt in both acidic and alkali water with pH ranging from 4 to 9.[15] Meanwhile, the domestic waste water with initial pH at 9.3 was significantly difference from the control group. This finding might possible to happen because waste water contained highly chemical substances from detergent and washing soap when the samples were taken. Burke *et al.*[16] revealed that the larvae of *Aedes sp* can survive either in sewage water or in septic tanks in Puerto Rico, so that, dengue transmission still took place even in dry season.

Furthermore, the imago of *Ae. aegypti* reared on sewage water had longevity of 9-11% in the third weeks in pH 8.7, but not in pH >9. In normal habitat, *Aedes* sp reared from clean or unpolluted water survived 100% in the first week and decreased in the second week until 4% left.

The observation in this study was conducted every week until 4 weeks old of the mosquitoes. During the observation, the mosquitoes were fed with sugar solution wetted in cotton that were replaced daily. In the first week, no dead pupa growing into imago were found. In the 2nd week of the observation, the number of dead mosquitoes were found in the pre-mature group media tested in the domestic waste water with pH of 7.7, 8.2, and 8.7. In the 2nd week, 80% of the control group survived. This was because the mosquitoes grown in the water media containing excessive chemical had organs that would not develop perfectly and would affect the lifespan of the mosquito to survive.

In addition, this study also observed residents' behavior in treating larva found in their container. They often dried the container containing larva by flushing the water directly into the sewage water. They were afraid that the larvae would become vectors and threat their family's health. The laboratory experiment of this study proved that the *Ae. aegypti* larvae still survived and even developed to be imago on household sewage water. Adult *Ae. aegypti*, reared in sewage water with pH interval of 7.7-8.7, could be a new vector that were able to transmit DHF to the community.

The results of measurements of the media temperature in the development of pre-adult mosquitoes range in temperature 24-27°C with an average temperature of the water medium is 25.3°C.[17] The measurement results from days 1 to 6 showed that the temperature of the room when the development of pre-adult mosquitoes ranges 25.3-26.6°C with an average of 25.7°C. This is in accordance with the optimal ambient temperature for mosquito development ranging 25-27°C.[18] This temperature is also in accordance with the optimum temperature for the development of pupae *Ae. aegypti* which ranges from 25-27℃. At the optimal temperature the male pupa requires an average developing time 1.9 days and female pupae require an average of 2.5 days. Some pupae can survive on water media with a temperature of 47 ° C for 5 minutes and 82% to 100% humidity can live at 4.5 ° C for 24 hours.[17]

Observation of imago has been carried out since the development of pupa became imago in each test. Mosquito *Ae. aegypti* usually live for 2 weeks, male mosquitoes usually only last until the age of 1 week, but there are also mosquitoes that last up to 2 months.[19] While the measurement of media temperature in the development of pre-adult mosquitoes ranged from 24-27℃ with an average temperature of water media that is 25.3 ° C. Based on WHO research, it is water temperature for optimum larval development.[20]

CONCLUSION

The *Ae. aegypti* larvae can survive and develop into mosquitos in not only pre-mature environment in clean water but also in household waste water environment. The habit of the people drain water containing larvae directly without considering to eradicate them causes *Ae. aegypti* larvae survive and potentially transform into DHF vectors, as they still live in waste water with pH of 8.7 (85%)

ACKNOWLEDGEMENTS

We would like deliver our appreciation and gratitude to the Faculty of Public Health, University of Diponegoro, Semarang, Central Java for the financial support and laboratory assistances who have helped the observation this study completed.

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