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The Equation Modeling of Environmental Risk Factors for The Existence of *Aedes aegypti* Larvae

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Abstract

Objective: This study aims to determine the risk equation model for the existence of *Aedes aegypti* larvae based on environmental risk factors like the water reservoirs characteristics (water source type, cover availability, colour, and location) and the water parameters of the breeding place (salinity, dissolved oxygen (DO), temperature, and pH).

Methods: The study design is observational analytic with a cross-sectional approach. The 86 houses in the endemic area of Martapura District were obtained using a simple random sampling method. Data were analyzed using Continuity Correction and Logistics Regression tests. The quality measurement of the equation model was using the Receiver Operating Curve (ROC) method for the discrimination test and using the Hosmer and Lameshow method for the calibration.

Results: Environmental factors that were significantly associated with the larvae's existence were the colour of the water reservoir (0.000), the cover availability (0.002), and the water source type (0.000). Factors that had no significant association were location (0.757), salinity (0.056), DO (0.669), temperature (0.223), and pH (0.156). The dominant factor which influenced the existence of larvae was the water source type (OR=16,777) after being controlled by colour (OR=14,448) and the cover availability (OR=8.022). The final equation model was $y = -4.676 + 2.820$ (water source type) + 2.671 (colour) + 2.082 (cover availability). The p-value of the Hosmer and Lameshow Test was 0.649 (>0.05). The Area under the Curve (AUC) was 0.945 (95% CI = 0.899-0.992; p-value = 0.000).

Conclusion: The equation model obtained has a good calibration and sufficient discrimination.

KEYWORDS: *Aedes aegypti*, dengue fever, environmental factors, equation model, larvae.

Introduction

Dengue fever has become a disease with extraordinary occurrences in several regions in Indonesia, one of which is in Banjar Regency, South Kalimantan Province. Martapura District is an endemic area with the highest incidence (47.8%).¹ Dengue fever is a problem that requires control measures so that the incidence of the disease does not continue to occur and increases, among others, by controlling the dengue fever vector, the *Aedes aegypti* mosquito. The existence of *Aedes aegypti* larvae are one indicator of the Dengue fever risk. Therefore, one of the efforts that can be done as action to control the dengue fever vector is to make the best model for predicting the presence of larvae.² The prediction model is in the form of a mathematical model that can provide an overview of the dominant factors causing the existence of larvae.

The prediction model for the presence of larvae can be developed based on the influencing

factors that cannot be separated from the epidemiological triangle. One of them is the environmental factor. Several previous studies have proven that there is a relationship between environmental factors including the characteristics of water reservoirs and water parameters of breeding sites with the presence of larvae.^{3,4} Characteristic factors of water reservoirs are the availability of cover, color, location, and type of water source.⁵ Water parameters of the breeding sites factors are temperature,⁶ salinity, pH^{7,8} and Dissolved Oxygen (DO).⁹⁻¹²

The distribution of the incidence of Dengue fever is influenced by the density of larvae.^{13,14} Previous research has shown that the presence of larvae can be influenced by the water quality of the breeding sites.¹⁵ The study result of Saleh, *et al.*,¹⁶ concluded that the eggs of the *Aedes aegypti* mosquito will develop under conditions of appropriate water parameter characteristics. Water quality is one of the important factors that determine the existence of life in water which is determined based on observations of various physical and chemical parameters.^{6,12,17} The results of Damanik's study in Hidayah, *et al.*,¹¹ showed that the water temperature of the breeding place was 27,6°C. This temperature is still in the optimum temperature range for the growth of *Aedes aegypti* larvae, resulting in the highest average number of larvae found in the breeding water. These results are in line with the research results of Saleh, *et al.*¹⁵ It is known that the average water temperature for *Aedes aegypti* mosquitoes is between 26,5°C to 29,3°C.

Several water chemical parameters have also been shown to affect the development of *Aedes aegypti* larvae such as salinity and Dissolved Oxygen (DO). Salinity (dissolved salt content) and DO (amount of dissolved oxygen) were shown to have a significant relationship with the growth of *Aedes aegypti* larvae.^{8,9}

Therefore, it is necessary to identify the characteristics of the reservoirs and the water parameters of the breeding places related to the presence of larvae so that a risk prediction model for the incidence of dengue fever can be made. This research is important as a basis for assessing the risk of dengue fever, especially during a pandemic as an optimization of the utilization of research and development results so that later they have the potential to be integrated into technology.

Method

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Study Design

This study is an analytical observational study with a cross-sectional approach that aims to create a predictive model of environmental factors that affect the presence of *Aedes aegypti* larvae. These factors then become research variables consisting of the characteristics of water reservoirs (color, presence of lids, type of water source, and location) and water parameters of breeding sites (salinity, dissolved oxygen, temperature, and pH). The cross-sectional study design model is presented in Figure 1.

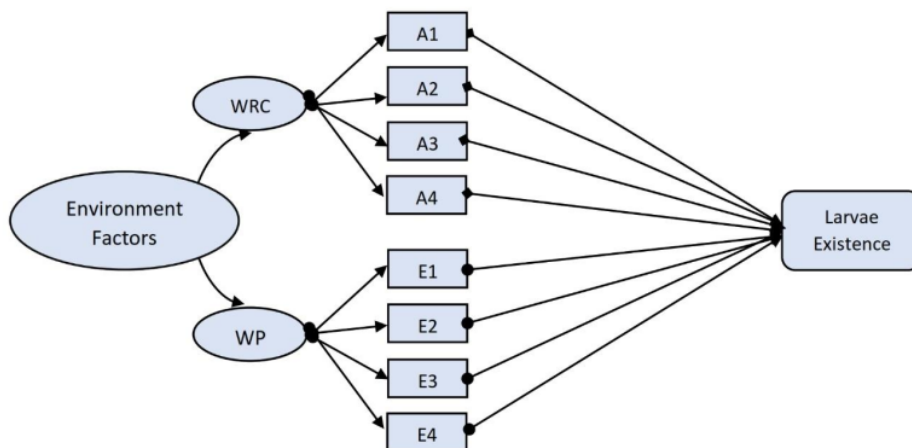
Subject

The study was conducted in Martapura District, Banjar Regency, South Kalimantan Province. It is an endemic area of dengue fever and has the highest incidence rate. The population of this study was all households in the Martapura District spread over 5 villages (Tanjung Rema Darat, Sekumpul, Sungai Paring, Indrasari, and Tanjung Rema) as many as 117,325 houses. The minimum sample size is 86 houses based on the results of Lemeshow's calculations and taken by simple random sampling.

Data Collection

The data collection instrument that has been used to obtain information about the variable characteristics of water reservoirs is a questionnaire. Data collection is done by observation.

Data collection instruments for water parameter variables in reservoirs are a 250 ml dipper, 600 ml glass/plastic storage container (bottle), a pipette, and an 8 L cooler box. The research material is H_2SO_4 as a sample preservative. The data collection was carried out by taking samples of water contained in the shelter using a dipper then putting it into a storage bottle and dripping 3-4 drops of H_2SO_4 using a dropper. Closing it tightly. The water sample is stored in a cooler box and then taken to the environmental laboratory of the Environmental Health Technical Center of South Kalimantan Province for measurements.



WRC: Water Reservoir Characteristics; **WP:** Water Parameters

A1: Colour; **A2:** Cover availability; **A3:** Water source type; **A4:** Location; **E1:** Salinity; **E2:** Dissolved Oxygen; **E3:** Temperature; **E4:** pH

Figure 1. Model of cross-sectional study design for environment risk factors that affect the larvae existence

Statistical Analysis

The data were analyzed using continuity correction with a 95% confidence level. Furthermore, multivariate analysis was performed using a logistic regression test. The prediction model was made by considering two assessments, the significance of the log-likelihood ratio ($p < 0.25$) and the p-wald value ($p < 0.05$).

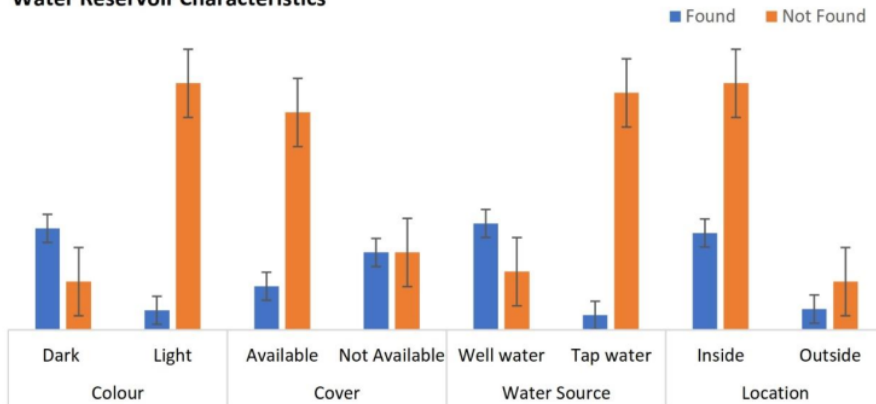
Model selection is done hierarchically. Eligible independent variables were entered into the model. Variables with insignificant p-wald were excluded from the model sequentially starting from the highest p-wald. Processing is carried out until the selected variable has a p-value of 0.05, which means that the variable is significantly related to the presence of larvae. After the best model is formed, the quality of the equation model is measured.

The quality of the model is assessed by calibration test and discrimination test. The calibration test used the Hosmer and Lameshow method and the discrimination test was using the Receiver Operating Curve (ROC) method by looking at the Area Under Curve (AUC) value. This research has obtained ethical clearance approval and research permit from the Health Research Ethics Commission of Sari Mulia University (No. 045/UE-UNISM/VII/2022).

Result

The results of the observation showed that, among 86 samples in Martapura District, on the predictor factors for the characteristics of water reservoirs, larvae were most commonly found in dark-colored reservoirs (24.4%), did not have a cap (18.6%), well water type (25.6%), and inside location (23.3%). In terms of water parameters, larvae were mostly not found in water with potential salinity (10.5%), potential dissolved oxygen (54.7%), optimum temperature (36.0%), and potential pH (23.3%) (Figure 2).

Water Reservoir Characteristics



Water Parameters

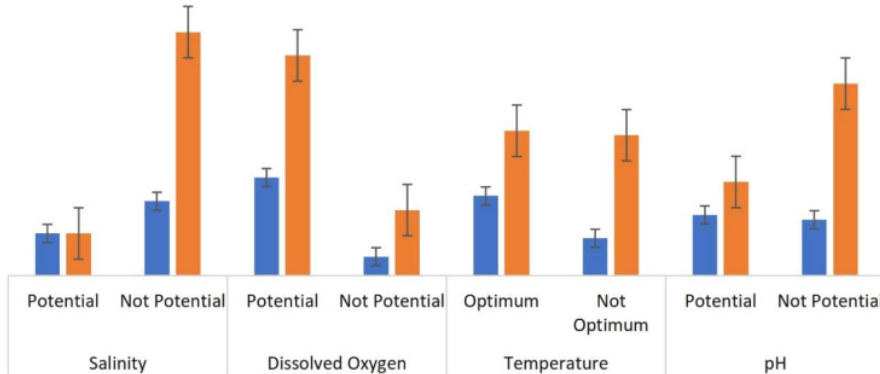


Figure 2. Observation result of larvae existence

Based on the results of the bivariate analysis, there were 6 variables with $p < 0.25$ which were then included in the multivariate logistic regression test. Two predictor variables that were not included in the multivariate test were location (p -value=0.757), and dissolved oxygen (p -value=0.669) (Table 1).

Tabel 1. The Association of Environmental Risk Factors with The Existence of *Aedes aegypti* Larvae

Environmental Risk Factors		RR (95% CI)	p-value	Remarks
Water Reservoir Characteristics				
Colour	Dark	0.037	0.000	Correlated
	Light	Missing ", "		
Cover availability	Available	0.200	0.002	Correlated
	Not Available			
Water source type	Well water type	0.033	0.000	Correlated
	Tap water type			
Location	Inside	0.784	0.757	Non-
	Outside	Missing ", "		Correlated
Water Parameters				
Salinity ^a	Potential	0.308	0.056	Non-
	Not Potential	Missing ", "		Correlated
Dissolved Oxygen (DO) ^b	Potential	0.639	0.669	Non-
	Not Potential	Missing ", "		Correlated
Temperature ^c	Optimum	0.486	0.223	Non-
	Not Optimum			Correlated
pH ^d	Potential	0.450	0.156	Non-
	Not Potential			Correlated

^a Potential (4-6 gr/l); Not Potential (<4 gr/l or >6 gr/l)

^b Potential (5.86-8.98 ppm); Not Potential (<5.86 ppm or >8.98 ppm)

^c Optimum (27-30 °C); Not Optimum (<27 °C or >30 °C)

^d Potential (≤7.5); Not Potential (>7.5)

In the results of the logistic regression test, it was found that in step 1st, 6 predictor variables were included. In step 2nd then 5 variables are entered by eliminating the temperature variable. In step 3rd, 4 variables were included by eliminating the pH variable. Furthermore, in the 4th step, 3 variables were entered by eliminating the salinity variable and the final model for predicting the risk of larvae was obtained.

Three variables have eligibility criteria as predictors of the presence of larvae. They are color, cover existence, and water source type (Table 2). Based on the results of the logistic regression test, the prediction model equation is $y = -4.676 + 2.820 (\text{water source type}) + 2.671 (\text{colour}) + 2.082 (\text{cover availability})$.

Prediction model quality assessment is carried out based on calibration and discrimination parameters. Hosmer and Lameshow test results are p-value = 0.649 (>0.05) which indicates that the prediction model has a good calibration (Table 3). The result of the discrimination test based on the Area Under Curve (AUC) is 0.945 (94.5%) which indicates a very good discrimination value (Figure 3). Secara keseluruhan, probabilitas model adalah 90% (Figure 4). A good model prediction should have a value above 0.5. A value less than 0.5 indicates the model is no better than random prediction.

Tabel 2. The Logistic Regression Model for Association of Environmental Risk Factors for The Existence of *Aedes aegypti* Larvae

Factors	B	p-value	OR	95% CI
1st STEP				
Colour	2.678	0.001	14.555	2.819-75.149
Cover existence	2.192	0.031	8.953	1.224-65.482
Water source type	3.142	0.001	23.140	3.545-151.054
Salinity	1.288	0.246	3.627	0.411-31.974
Temperature	-0.057	0.954	0.944	0.135-6.602
pH	-0.888	0.370	0.412	0.059-2.865
2nd STEP				
Colour	2.678	0.001	14.555	2.818-75.164
Cover Availability	2.163	0.014	8.694	1.561-48.412
Water source type	3.141	0.001	23.136	3.541-151.152
Salinity	1.290	0.245	3.634	0.413-31.976
pH	-0.904	0.342	0.405	0.063-2.613
3rd STEP				
Colour	2.558	0.002	12.910	2.597-64.176
Cover Availability	2.006	0.019	7.432	1.387-39.827
Water source type	2.924	0.001	18.620	3.305-104.900
Salinity	0.855	0.379	2.351	0.351-15.757
4th STEP				
Colour	2.671	0.001	14.448	2.933 – 71.163
Cover existence	2.082	0.014	8.022	1.532 – 42.006
Water source type	2.820	0.001	16.777	3.183 – 88.442
Constant	-4.676			

B = Beta Coefficient; OR = Odd Ratio; 95% CI: Confident Interval 95% (0.05)

Tabel 3. Result of Hosmer and Lameshow Test

Sp. STEP	Chi-square	p-value
1 st	6.145	0.407
2 nd	5.074	0.534
3 rd	3.794	0.705
4 th	2.473	0.649

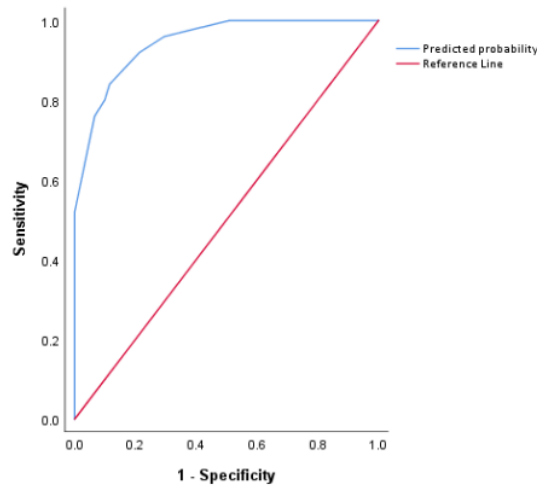


Figure 3. The curve of AUC ROC analysis (0.945; 95% CI = 0.899-0.992; p-value = 0.000) for discrimination test of the equation modeling of environmental risk factors for the existence of larvae.

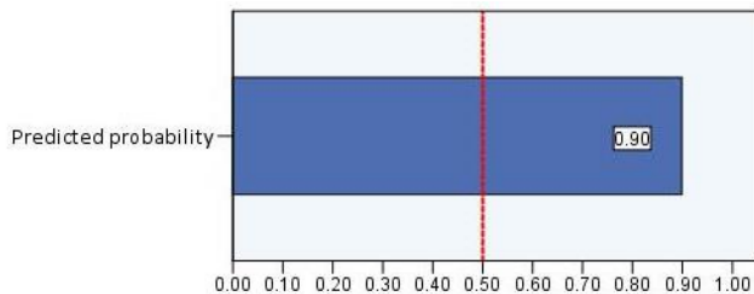


Figure 4. Overall model quality

Discussion

Based on the study of the epidemiological approach in examining the spread of a disease, this research focuses on environmental factors. The spread of dengue fever is strongly influenced by the potential environment for *Aedes aegypti* breeding which is indicated by the presence of larvae.^{15,16}

The results showed that several characteristics of the water reservoirs proved to have a significant effect on the presence of larvae, including dark-colored reservoirs. This is in line with Torrisi and Hoback's research¹⁸, it is known that dark cup color can promote oviposition. However, these results are in contrast to Affiandy's research¹⁹, most *Aedes aegypti* larvae were found in light-colored containers (31.25%). In the research sample, most of them were light in color, and in fact, they still found *Aedes aegypti* mosquito eggs. This shows that there are other influencing factors, such as the container material, water cleanliness, and the frequency of draining the reservoir water.^{20,21,22}

In addition, this study also proves that there is a significant relationship between the presence of larvae and the characteristics of containers that do not have lids. This is because mosquitoes will enter to place their eggs on the walls of the container easily. Coarse-textured container walls are preferred by mosquitoes to lay their eggs²². Breeding water sources have also shown to be related to the presence of larvae. This result is related to the availability of microorganisms as food during the development of larvae^{5,23}. Well water is proven to be preferred by mosquitoes for breeding⁵, it tends to be clear, low salinity, neutral water pH 6-8, and contains microbes as the main food source for larvae²³.

The chemical and physical parameters of water studied in this study also proved to be significantly related to the presence of larvae. Salinity or salt content of the brooding water can affect the rate of reproduction of *Aedes aegypti* larvae, especially at levels of 12-18‰²³. In addition, water parameters that are proven to be significantly related are temperature and pH. The results of this study are in line with several previous studies such as in Brazil¹⁴, Tanzania^{15,21}, Iran⁷, and Colombia^{24,25}.

Temperature can affect the development of larvae. It will develop well at the optimum temperature but take longer at low temperatures. This affects the physiological processes of the larvae^{10,11,23}. The relationship between pH is where pH affects the levels of O₂ and CO in the water. It affects the formation of the cytochrome oxidation enzymes of *Aedes aegypti* larvae. The development of eggs to become larvae and then to become adult mosquitoes is at a pH of 4-9^{13,17,26,27}.

Among the factors above, there are three key factors that are the determining factors in the model obtained (water source type, color, and cover availability). When using the equation model, the minimum score would be -4.676 (when only negative predictors are present) and the maximum score would be 2.897 (when only positive predictors are present). The risk presentation ranges from 0.92% to 94.8%. So far, previous studies have made models to predict the incidence of dengue fever²⁸⁻³⁰ as well as to predict the type of dengue fever³¹, but a new larva prediction model is now being carried out. Even though the overall quality of the model is good, this equation model needs to be tested further on populations, both during epidemics and non-epidemic times.

Conclusions

The equation model obtained has a good calibration and has sufficient discrimination in predicting the presence of *Aedes aegypti* larvae.

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