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Table of Contents

Timing and Determinants of Tuberculosis Treatment Interruption in Nairobi County, Kenya Violet Jephumba, Simon Karanja, Evans Amukoye, Lawrence Muthami, Hillary Kipruto	PDF 203-212
Prevalence and Determinants of Anemia in Pregnancy, Sana'a, Yemen Yousef M. Alfiah, Iman H. Wahdan, Ali A. Hasab, Dalia I. Tayel	PDF 213-220
Knowledge, Attitude and Practice of Cervical Cancer Screening through Visual Inspection with Acetic Acid Susmita Kar, Md. Kapil Ahmed	PDF 221-230
The Effectiveness of Counseling through Vasectomy Module in North Kolaka, Indonesia Askrening Askrening, Hendra Yulita	PDF 231-236
Container Positivity and Larva Distribution Based on the Container Characteristics Wanti Wanti, Ririh Yudhastuti, Subagyo Yotopranoto, Hari B. Notobroto, Sri Subekti, Sitti R. Umniati	PDF 237-242
Dietary Diversity Practice and Associated Factors Among Infants and Young Children in Haramaya Town, Ethiopia Berhe Gebremichael, Gudina Egata, Nega Assefa	PDF 243-250
Developing Primary Liaison Psychiatric Services for HIV/AIDS Patients in Community Ahsan Ahsan, Retno Lestari	PDF 251-256
Risky Sexual Behavior and Associated Factors Among High School Students in Gondar City, Northwest Ethiopia Abebaw Wasie Kasahun, Mezgebu Yitayal, Tadele Girum, Bekri Mohammed	PDF 257-265
Incidence and Predictors of Tuberculosis among Adult PLWHA at Public Health Facilities of Hawassa City Henok Bekele, Mesfin Kote, Aman Yesuf, Tadele Girum	PDF 266-274
The Effect of Satisfaction, Perceived Value, Image, and Perceived Sacrifice on Public Healthcare Service Institution's Patient Loyalty Medi Yarmen	PDF 275-282



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Nama : Dr. Santi Martini, dr. M.Kes
NIP : 196609271997022001
Pangkat/Golongan : Pembina / Gol. (IV/a)
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Dengan ini menerangkan bahwa :

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NIP : 196506251992031002
Pangkat/Golongan : Pembina (Gol. IV/a)
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Demikian surat keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional Guru Besar.

Surabaya, 24 April 2023

Dr. Santi Martini, dr. M.Kes
196609271997022001



Container Positivity and Larva Distribution Based on the Container Characteristics

Wanti¹, Ririh Yudhastuti², Subagyo Yotopranoto³, Hari B. Notobroto⁴, Sri Subekti⁵, Sitti R. Umniati⁶

^{1,2,4} Faculty of Public Health, UNAIR, Surabaya, Indonesia

³ Faculty of Medicine, UNAIR, Surabaya, Indonesia

⁵ Faculty of Fisheries and Marine, UNAIR, Surabaya, Indonesia

⁶ Faculty of Medicine, UGM, Yogyakarta, Indonesia

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ABSTRACT

Dengue Haemorrhagic Fever (DHF) prevention and control program has been conducted in Kupang City but DHF Incidence Rate (IR) is always high and exceeds the national average. Calculating the density of larvae and pupae is a good choice in calculating DHF mosquitos' density because larvae capture is easier than mosquitoes, and pupa is the nearest stage with adult mosquitoes so can better represent actual mosquito density. This observational study used cross sectional study design was conducted in Kupang City as many 24 urban villages or 480 houses during the rainy and dry season and all containers inside and outside homes selected been observed. Larvae and pupae were collected using gamadotik then identified. Analyzed data were using multiple regression logistic test, independent t test and Anova test. This study found 781 containers with the equation model of container positivity = 2,975 lid - 0.192 material + 0.781 type - 3,706, with the most influential variable is the container lid (OR 19.5). *Ae. Aegypti*, *Ae. Albopictus* and *Culex* can be found in water containers both inside and outside homes. The presence of larvae can be prevented either by doing closed and drain the container regularly and bury or drying container that is not used anymore. As limitation of water supply so other solutions need to be taken such as by sprinkling temefos into container with closed tightly to reduce the container positivity 19.5 times. For greater container can be done by water draining or reuse used container outside the home into a place to plant flowers or plants or become temporary garbage.

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Corresponding Author:

Wanti,

Faculty of Public Health,

UNAIR, Surabaya, Indonesia.

Email: trivena78@yahoo.com

1. INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is a vector borne disease that contributes to disease burden, high mortality, poverty and social burden in the tropics and many days lost due to DHF each year [1]-[5]. As many as 70% of dengue cases worldwide live in Southeast Asia and the Western Pacific including in Indonesia [4]. DHF cases in Indonesia grew from 58 cases and 2 cities in 1968 to 100,347 and 433 cities / districts in 2014 [6]-[8].

Nusa Tenggara Timur (NTT) province including DHF endemic with Incidence Rate (IR) from 2000-2014 ranged from 0.6 to 37.1 cases per 100,000 population, and this figure is lower than national IR ranging from 10.2 to 71.8 cases per 100,000 population. This is in contrast to Kupang City as the capital of NTT province which IR from 1999-2015 ranged from 11.1 to 266.5 cases per 100,000 population and this IR is always the highest in NTT and also always higher than the national average.

DHF prevention and control program has been conducted in Kupang City until 2015 with activities such as mosquito nest eradication, epidemiological investigation, abatement, fogging and counselling [9], but DHF IR is always high and exceeds the national average, and the larval free rate are always low and do not follow the rainfall pattern [10]. Previous research has found that the percentage of water reservoirs in larval positive is greater in non-endemic areas than in dengue endemic areas [11], but that study was conducted only during the dry season so it is important to examine whether the season affects the presence of larvae and Pupa in water containers.

Calculating the density of larvae and pupae is a good choice in calculating DHF mosquitoes density because larvae capture is easier than mosquitoes, and pupae is the nearest stage with adult mosquitoes so pupa density can better represent actual mosquito density. Given the importance role of water container in the presence of *Aedes* sp, this study will analyze the effect of container characteristics on the container positivity (the presence of larvae and pupae in container) and analyze the difference of average mosquito larvae species based on container characteristics. Until now there is no proper medicine that can be used for dengue disease [12], so the knowledge about the density of larvae and pupae and risk factors will be very helpful in breaking the chain of transmission of DHF by predicting and depressing the larval density of larvae and pupae.

2. METHOD

This study was observational research using cross sectional study design. The study was conducted in 12 urban villages during the dry season and 12 urban villages during the rainy season that spread across 6 sub-districts in Kupang City, NTT Province. Each urban village took 20 homes to sample the research so that the total sample of the study was 480 houses. Samples for container are all containers in and out of homes in 20 houses in 24 selected urban villages. Figure 1 shows the tool for collecting larvae and pupae that named Gamadotik.

Independent variables in this research are container type (bathtub, drum, reservoir, dispenser, bucket, and tank profile), container location (outer and in house), container material (cement, iron, ceramic and plastic), container lid condition (open, semi-open and closed), container color (dark and light) and season (dry and rainy season), while the dependent variable is the presence of larvae and pupa in container (container positivity). The data obtained are primary data that is obtained by direct observation of container conditions conducted in the dry season and then the observations are repeated in the rainy season. In addition, all larvae and pupae in container in homes selected were collected using gamadotik ie tools for collection of all larvae and pupae made by Faculty of Medicine UGM. The larvae were then identified in the entomology laboratory using a larval identification guide [13].



Figure 1. Gamadotik (Tool for collecting all larvae and pupae in container)

Data of container characteristic and container positivity were then presented in tabular form. All data including categorical data so were analyzed using multiple regression logistic test to make predictive model of container positivity. Distribution of larvae species were made in tabular form, then the difference of average number of larvae species in each container was tested by independent t test and Anova test.

3. RESULTS

3.1. Results

The univariate analysis showed that the higher container with larvae and pupae were found in drum, out-of-home container, iron-filled container, open or semi open lid container, dark-colored container and in the rainy season. Detailed distribution of container with larvae and pupa can be seen in Table 1.

Table 1. The Relation of Container Positivity with The Research Variables

Condition of Container		Larvae/Pupae (-)	Larvae/Pupae (+)	Total	P value	OR	CI 95 %
Type	Not a drum	385 (71.5%)	155 (28.7%)	540 (100%)	0.000	2.051	1.497 – 2.810
	Drum	132 (55.2%)	109 (45.2%)	241 (100)			
Location	In house	309 (67.0%)	152 (33.0%)	461 (100)	0.556	1.095	0.810 – 1.479
	Outer house	208 (65.0%)	112 (35.0%)	320 (100%)			
Material	Not iron	391 (71.1%)	159 (28.9%)	550 (100%)	0.000	2.049	1.491 – 2.816
	Iron	126 (54.5%)	105 (45.5%)	231 (100%)			
Lid	Tightly closed	74 (97.4%)	2 (2.6%)	76 (100%)	0,000	21.883	5.328 – 89.881
	Open/Half closed	443 (62.8%)	262 (37.2%)	705 (100%)			
Color	Bright colors	121 (69.5%)	53 (30.5%)	174 (100%)	0.287	1.216	0.846 – 1.750
	Dark colors	398 (65.6%)	209 (34.4%)	607 (100%)			
Season	Rainy	212 (64.8%)	115 (35.2%)	327 (100%)	0.494	0.901	0.667 – 1.215
	Dry	305 (67.2%)	149 (32.8%)	454 (100%)			
Total		517 (66.2%)	264 (33.8%)	781 (100%)			

In bivariate analysis there were only 3 variables related to container positivity because it has p value <0.25 ie container type, container material and container cap. There are only three variables are included in multivariate analysis, as shown in Table 1.

The results of multivariate analysis showed that from 3 variables analyzed only one of P value <0.05 that is container lid condition, which type and container material have p value > 0.05 so type and container material are excluded and starting from a higher p value and because there is a change of odd ratio (OR) >10% so that the two variables are re-entered to obtain the final result as in Table 2.

Table 2. Multiple Regression Logistic Result of Research Variables

Variables in the Equation	B	S.E.	Wald	Df	Sig.	Exp(B)	95 % CI
Lid	2.975	0.724	16.872	1	0.000	19.597	4.738 - 81.054
Material	-0.192	0.657	0.086	1	0.770	0.825	0.228 - 2.989
Type	0.781	0.651	1.439	1	0.230	2.184	0.610 - 7.823
Constant	-3.706	0.720	26.499	1	0.000	0.025	

Table 2 shows the model of the final equation: Container Positivity = 2,975 lid container - 0.192 container material + 0.781 container type - 3,706. The presence of larvae and pupae or container positivity influenced by three variables simultaneously equal to 66.2%, meaning 33.8% of the existence of larvae and pupa influenced by the existence of other variables not examined in this study. The most influential variable in this research is the presence of container cap with OR 19.595 meant open or half closed container gives the possibility of a container for positive larvae and pupae 19.595 times larger than closed container.

This research also identifies larvae species and its distribution based on container characteristic. Not all larvae and pupae could be identified in this research, and most identified mosquitoes (96.4%) are *Aedes aegypti* (*Ae. Aegypti*), while *Ae. albopictus* and *Culex sp* is only 1.2% and 2.3% respectively, as seen Table 3. Mostly *Ae. Aegypti* and *Culex sp* are found in bathtubs, while *Ae. albopictus* in drums. Anova test results show that there is an average difference of Larvae *Ae. Aegypti* between container that is between bathtub and tank profile with p value 0.0045. Table 3 shows that *Ae. Aegypti* is more common in container in the house, while *Ae. albopictus* and *Culex sp* are commonly found in outside container. Based on independent t test there is no difference of average number of larvae *Ae. Aegypti* between container types with p value 0.49. As for the average *Ae. Albopictus* there is a difference between being located inside the house and outside the house with a p value of 0.05. The average of *Culex sp* larvae is also no different between containers with p value 0.25.

Table 3 also shows that mosquito larvae are commonly found in the container of cement. Specifically *Ae. Aegypti* and *Culex sp* are found in the container of cement, while *Ae. Albopictus* and *Culex* are commonly found in iron material. Based on the Anova test, no difference average *Ae. Aegypti* larvae were found between different material with p value 0.08. While the average larvae *Ae. Albopictus* there is a

difference between the cement material and iron material with p value of 0.05. The three species of mosquitoes are most commonly found in opened containers, whereas confined containers do not find any mosquito larvae except *Ae. Aegypti*. Anova one way test obtained value 0.004 which means there is a difference in the average larvae *Ae. Aegypti* between closed and opened container, while the average difference for *Ae. Albopictus* and *Culex sp* based on the lid condition is not found. Species *Ae. Aegypti* and *Ae. Albopictus* are more common in the dry season, while *Culex sp* is more common in the rainy season. Based on the result of Independent T Test it is found that there is no difference of average of *Ae. Aegypti* larvae in the dry season and rainy with p value 0.381, while for the average larvae *Ae. Albopictus* and *Culex sp* there is a difference between rainy season and dry season with p value of 0.05 and 0.017.

Table 3. Distribution of Mosquito Larvae Species by Container Type

Container	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Culex sp</i>	Total	
Type	Bathub	8,908 (60.9%)	32 (17.2%)	262 (73.8%)	9,202 (60.6%)
	Drum	5,188 (35.5%)	152 (81.7%)	92 (25.9%)	5,432 (35.8%)
	Reservoir	275 (1.9%)	3 (1.6%)	0 (0.0%)	278 (1.8%)
	Dispenser	6 (0.0%)	0 (0.0%)	0 (0.0%)	6 (0.0%)
	Bucket	256 (1.7%)	0 (0.0%)	0 (0.0%)	256 (1.7%)
Location	Profil tank	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	In house	9,094 (62.1%)	32 (17%)	118 (33.2%)	9,244 (60.9%)
Material	Outer house	5,539 (37.9%)	155 (83%)	236 (66.8%)	5,930 (39.9%)
	Cement	7281 (49.8%)	35 (18.7%)	131 (37%)	7447 (49.1%)
	Iron	4997 (34.1%)	152 (81.3%)	71 (20.1%)	5220 (34.4%)
Cover	Ceramic	1454 (9.9%)	0 (0%)	24 (6.8%)	1478 (9.7%)
	Plastic	901 (6.2%)	0 (0%)	128 (36.2%)	1029 (6.8%)
	Opened	12,113 (82.8%)	136 (72.7%)	239 (67.5%)	12,488 (82.3%)
	Half opened	2,358 (16.1%)	51 (27.3%)	115 (32.5%)	2,524 (16.6%)
Season	Closed	162 (1.1%)	0 (0.0%)	0 (0.0%)	162 (1.1%)
	Dry	9,099 (62.2%)	187 (100%)	10 (2.8%)	9,296 (61.3%)
	Rainy	5,534 (37.8%)	0 (0.0%)	344 (97.2%)	5,878 (38.7%)
Total	14.633 (100%)	187 (100%)	354 (100%)	15.174 (100%)	

3.2. Discussion

This research found that the larvae/pupae positive container was less than that of the negative larvae/pupae container with 33.8% Container Index (CI) where the CI outdoors (35%) was higher than inside the house (33%). This CI number in Kupang City is similar to CI in Tammamaung Makasar Village which obtains CI of 32.6% [14], but this CI is higher than in Banjarbaru as a city that is also endemic to DHF (28% CI), and CI in the home is also higher in Kupang City than in Banjarbaru which is only 28.7%, as well as CI drum container higher in Kupang City than in Banjarbaru which is only 29.4% [15].

Seen from the CI, Kupang City is a high-risk area for Dengue transmission [15] because $CI \leq 10\%$ means an area will be safe from the risk of Dengue transmission. The high CI shows the density of mosquitoes is also high and this of course will increase the risk of Dengue transmission in Kupang City, for it needs an equation model to predict the presence of larvae and pupae in a container. Of the 6 independent variables studied, it was found that when the bivariate analysis only got three variables that p value < 0.25 so that only 3 variables can be included in multivariate analysis that is container type, container material and container lid. After multivariate analysis finally got the equation model:

$$\text{Container Positivity} = 2,975 \text{ lid container} - 0.192 \text{ container material} + 0.781 \text{ container type} - 3,706$$

The container positivity or the existence of larvae and pupae in container are influenced by three variables simultaneously equal to 66.5%. The most influential variable is the presence of container cap with OR 19.6 or open or half closed container gives the possibility of a container for positive larvae and pupae 19.6 times larger than closed container. The equation model shows that 33.5% of the existence of larvae and pupae are influenced by other variables not examined in this research. Other variables include the frequency of water filling and water volume [16], or possibly other variables such as conductivity, total dissolved solids (TDS), alkalinity, salinity, turbidity and the water's calcium content in the landfill [16]. In addition the condition of the water container as a breeding ground for *Aedes sp* mosquitoes and the quality of housing such as house spacing, lighting, and construction of buildings and the condition of house sanitation also play a role in providing breeding grounds and the presence of *Aedes sp* [13],[17],[18].

The study found the *Aedes* and *Culex* larvae distributed in baths and drums from cement, iron, ceramics and plastic both inside and outside the house with *Ae. Aegypti* percentages 96.4%, *Ae. Albopictus*

1.2% and *Culex sp* 2.3%. Distinct distribution of *Ae. Aegypti* and *Culex tritaeniorhynchus* is the temperature, rainfall and topography of the region, whereas TDS and pH of water are not related to the existence of both species [19]. This is in contrast to previous theories that *Aedes* enjoys living in clear water, and *Ae. Albopictus* live outside the home in the garden and around the house [13]. *Culex* is also used to living in dirty stagnant water, but in this study *Culex* is also found in bathtubs and drums commonly used to accommodate clean water. *Culex* prefers to breed in dirty and stagnant water, but this study of *Culex* is also found in containers for daily necessities of bathrooms and drums, in which containers are also found *Aedes*. This is likely due to water limitations so that the bathroom and drum are rarely cleaned and the water tends to be dirtier than the more buckets that are easier to clean.

Recent research has found that *Ae. Aegypti* larvae can also live in contaminated water such as sewer water [20]. This study found the existence of *Aedes* and *Culex* in one container that is in the drum and bathtub both inside and outside because the possibility of water in the container is very dirty due to water limitations so rarely drained and there is also a drum and bathtub that is not used anymore but Still contains water so that both genera of mosquito larvae can still live and breed,

Ability *Ae. aegypti* to live in polluted water is also supported by previous research that mention *Ae. aegypti* is most dead in taps water and more able to live in sewer water with total growth of larvae and pupae in sewer water of 5.33% compared to dug well water, rain water and taps water [20]. The existence of mikroorganisme as a food source of larvae in sewer water is likely to support the high survival and larval growth rate so that on the second day the larvae can grow into pupae in sewer water [20].

Similarly, another research found that the ability of *Aedes sp* eggs to hatch and the ability of *Aedes sp* larvae live on sewer water is higher than that of dug wells and taps water, however *Aedes sp* does not survive in water polluted with soapy water [21]. Previous research has also found a variety of species of mosquito larvae in a natural and artificial container that is *Ae. Aegypti*, *Ae. Albopictus*, *Culex quenegefasciatus*, *Armigeres subalbarus*, *Toxorhynchites sp* and *Lutzia sp* [22] and there explained that the larval density negatively associated with the conductivity and pH. While another study explained the existence of larvae associated with temperature [23].

With the discovery of larvae of *Aedes* and *Culex* in container for daily need shows that should the high presence of larvae in these containers can be prevented either by doing 3M (*Menguras, Menutup, Mengubur* *English: drain, seal, bury) is closed and drain the container regularly and bury or drying container that is not used anymore. Keep in mind in the water drain should be done while brushing against the inner wall of the container so that the mosquito eggs that are attached can be loose and wasted along the flow of water. Kupang city has limited water supply so that the depletion of container activities can not be done on a regular action, so other solutions need to be taken, for example by sprinkling temefos into container coupled with tightly closing the landfill. It is also based on the results of this study which showed in Kupang City closed container is only 2.6% contained larvae and pupae while the the opened or half closed 36.9%. The cover has OR of 19.4 so as to seal off will reduce the risk of the presence of larvae and pupae in the container by 19.4 times.

Container drum also has the percentage of positive larvae and pupae are greater than not drum with an OR of 2.2, which means when the drum no longer used as a water reservoir again or when the drum as a whole are firmly closed, it can reduce the risk of the presence of larvae and pupae in Kupang City 2.2 times than when the drum is still in use or still not closed tightly. In this study because the existing containers are mostly large so that if not used again it is not possible to burial but water draining or reuse used container can be a good choice to solve this problem, such as used drums used to be flower pots or trash can, used bath tub outside the home can also reused into a place to plant flowers or plants and become temporary garbage or fish pond.

4. CONCLUSION

The final equation model of Container Positivity = 2,975 lid container - 0.192 container material + 0.781 container type - 3,706. The presence of larvae can be prevented either by doing closed and drain the container regularly and bury or drying container that is not used anymore. Keep in mind in the water drain should be done while brushing against the inner wall of the container so that the mosquito eggs that are attached can be loose and wasted along the flow of water. As limitation of water supply so that the depletion of container activities can not be done on a regular action, so other solutions need to be taken such as by sprinkling temefos into container coupled with tightly closing the container to reduce the risk of the presence of larvae and pupae in the container 19.6 times. It is also need to manage the drum by closed firmly or reused used drum to reduce the risk of the presence of larvae and pupae 2.2 times. For greater container like bath tub can be done by water draining or reuse used bath tub outside the home into a place to plant flowers or plants or become temporary garbage.

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