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The Effectiveness of Net to Reduce the Entomological Indices in Dengue-Endemic Areas in Balikpapan, Indonesia

Ike Helena Febriana[1,2], Ansariadi Ansariadi[1]*, Hasanuddin Ishak[3], Ida Leida Maria[1], Ridwan Aminuddin[3], Agnees Pamantouw[2]

[1]Departement of Epidemiology, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia; [2]Departement of Disease, Prevention and Control, Balikpapan City Health Office, Balikpapan, Indonesia; [3]Departement of Environmental Health, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia

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***Correspondence:** Ansariadi Ansariadi, Department of Epidemiology, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia. E-mail: ansariadi@unhas.ac.id
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Abstract

BACKGROUND: Drums and cisterns are ubiquitous water storages in Indonesian households, seldom being drained and left open to create access for the rainwater, providing a favorable breeding site for dengue vector. The bigger the container, the more it produces immature mosquitoes that are soon to be mature, increasing the entomological indices and raising the potency of cases in the area. Previous studies revealed that the net covering the reservoir was able to effectively protect the water from mosquito oviposition; therefore, a modification of the net was made.

AIM: The aim of this study is to discover whether the net as a cover for water containers is effective in reducing the entomological indices in dengue-endemic areas.

METHODS: The quasi-experimental study with pretest and posttest control group design, involved 3 intervention and 3 control clusters, 150 houses which have 672 water-holding containers with 116 large containers were intervened with non-insecticide tulle nets for 3 months. The larval presence data were performed by larval survey.

RESULTS: It revealed that net reduced the container index (CI) in intervened large containers 18%–84% as well as the environment entomological indices in general in study areas: CI decreased 75%–79%, house index decreased 65%–70%, and Breteau index decreased 75.5%–78.7%, while Free Larva Index rose 73.7%–88%.

CONCLUSIONS: The nets had lowered the CI in the intervened large container and affected the entomological indices of the surrounding environment, by blocking the mosquitos-water contact and preventing the young mosquitos that had developed in the containers from flying out.

Introduction

Dengue is a major public health problem in tropical and subtropical regions that infects all ages. It is estimated that 390 million dengue virus infections occur per year in 129 countries, and 70% of the burden is in the Asian region [1]. In Indonesia, cases of dengue hemorrhagic fever in 2020 up to the week 49th were recorded as many as 95,893 with 661 deaths; the largest morbidity occurred at school age 5–14 years (33.97%) and productive age 15–44 years (37.45%) [2].

Preventing and reducing transmission of dengue virus entirely depends on controlling mosquito vectors or breaking human-vector contact [3]; the latter is difficult. Vector control targets the eradication of larvae's breeding sites (aquatic phase), because in this phase, eradication of mosquitoes is easier than when mosquitoes are adults and can move freely. To reduce the transmission of dengue cases, the vector index needs to be low [4]. The Indonesian Ministry of Health has developed an *Aedes aegypti* vector control program with the 3M Plus (Menguras, Menutup, Mengubur plus Menabur Larvasida/to drain, to lid, to burry used goods or to recycle plus sow the larvicide). The 3MPlus becomes jargon in Indonesia to call the activities which eradicate breeding places of immature mosquitoes that transmit DHF by draining and lid the water containers, burying/recycling used goods, plus sow the larvicide [5].

However, vector control methods are a hitherto problem in Indonesia. Many aspects make vector control not work out in some areas due to the 5-year cycle, geographical conditions (temperature and weather), and community effectiveness of larvicides (wrong dose, wrong storage, and wrong application) [6]. Meanwhile, from interviews with Puskesmas (Community Health Center) officers regarding vector control constraints in their area, they said that limited health cadres, refusal to use larvicides, and community behavior that did not comply with 3M Plus made cases of dengue hemorrhagic fever easy to rise.

Fluctuations in dengue cases still follow the seasons. Community's habits regarding season create problems to vector control programs, for instance, collect rainwater during the rainy. The water reservoir is left open by the residents; hence, whenever it rains, the water can go directly into the reservoir without having

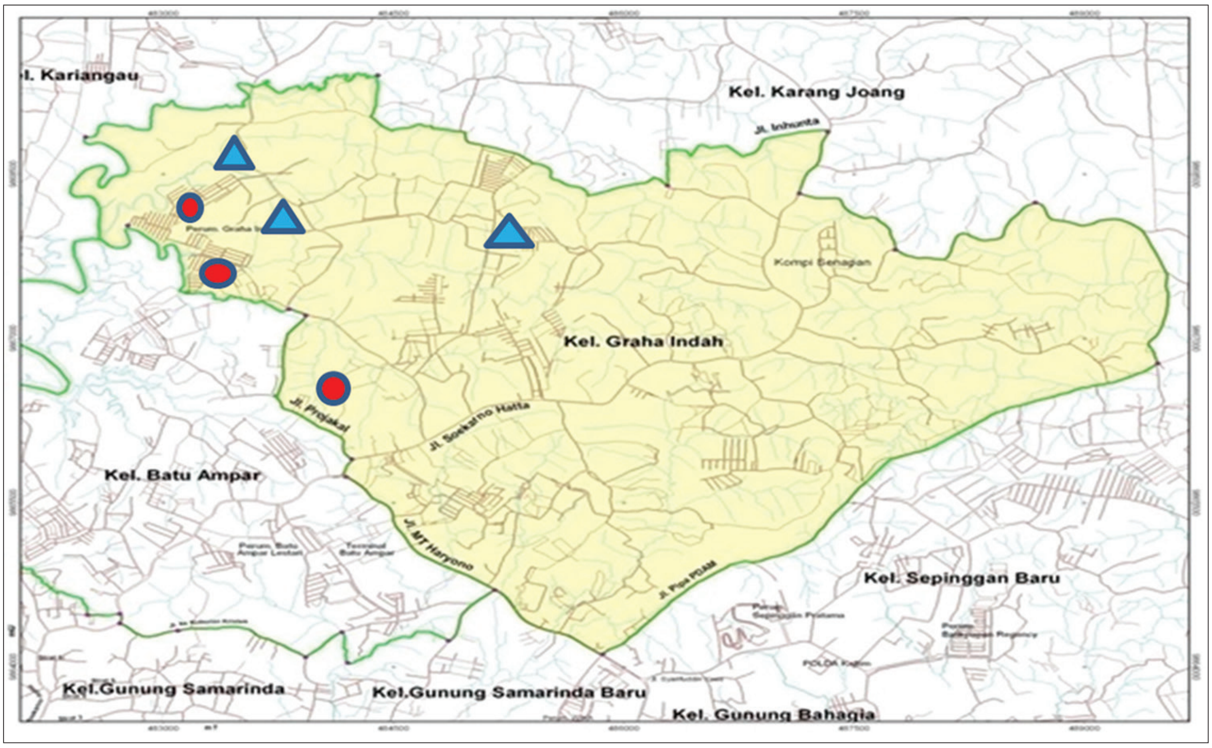

Figure 1: Map of Graha Indah subdistrict, North Balikpapan district, Balikpapan city. Source: Graha Indah Community Health Center, 2021.: net clusters, : control clusters

to open the reservoir lid, especially when they are not at home. The open water container creates breeding place for female *Aedes aegypti* mosquitoes to infest the eggs [7]. Meanwhile, surmise over the temephos resistance which has been used for 40 years in vector control programs in Indonesia [8], [9] and the alleged decrease in efficacy due to dilution by high water turnover in the rainy season are other problems from the internal program, besides the rejection temephos by community because of its smell. Therefore, a new method is needed to be an alternative solution in controlling the dengue vector that is accepted and can be practiced more broadly by the community so the cases can be reduced.

Covering domestic water storage containers is recommended by the WHO [1]; this is a low-cost and effective vector control to prevent mosquitoes from contacting water. In controlling vector, we need to limit the contact of adult mosquitoes with water reservoirs to prevent oviposition; hence, the water-holding container does not become a breeding place. Krueger *et al*. found that windows and covers for household water containers with insecticide nets can reduce the Breteau index (BI), reduce dengue vector density to a lower level, and potentially affect dengue disease transmission [10], [11].

Inspired by Krueger's research, the net method that covers the water containers was adopted with some modifications to be used as a method for controlling dengue vectors in dengue-endemic areas in Indonesia. This study aims to see the effectiveness of using non-insecticide nets to cover water reservoirs by decreasing the entomological indices in dengue hemorrhagic fever endemic areas in Indonesia.

Methods

This is a study with a quasi-experimental approach, with pre-test and post-test measurements. The sampling method used random sampling to determine the samples but used purposive sampling as the single-stage cluster sampling to determine houses instead of random sampling because of the need to create clusters in the study area. A cluster is a population with homogeneous characteristics and has the same opportunity to be part of the sample [12]. If there are one or more houses that do not meet the inclusion criteria, then the cluster is shifted until a homogeneous sample cluster is formed. It is intended to limit the movement of the *Aedes aegypti* mosquito, which can fly to lay eggs up to 320 m across the urban area [13], expected to minimize bias in larval surveys.

The device in this study is nets made of tulle, with pore diameter <2 mm, sewn circular on the edges (round shape), 120 cm in diameter, and has rope along the edge of the net as fixation. To avoiding ethics issue, even though the control clusters gave no information about the study and obtained no specific intervention, they still went with what they usually do on a daily basis for vector control.

Population and sample

The study was conducted in Graha Indah subdistrict, Balikpapan city, East Kalimantan province, Indonesia.

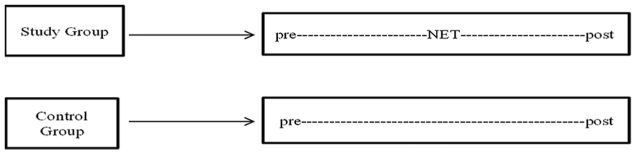

Figure 2: Design of study

The population of this research is all the Rukun Tetangga (RT) (neighborhood) in the Graha Indah subdistrict which consists of 73 RTs. RT is a small group of households with a maximum number of 150–250 people based on the Indonesian National Standard for Housing Planning (SNI 03-1733-2004) [14]. The sample of this study consisted of six clusters of RTs selected randomly, three study clusters, and three control clusters (Figure 2). The cluster formed consists of equal 25 houses so that there was no difference in the number of houses in each study area. We chose 25 houses each, to create a homogeneous cluster, and the RT with the least number of buildings could still form a cluster of the same number of houses as other clusters because the premises in each RT varied from 60 to 150 including empty houses, abandoned buildings, and families in COVID-19 isolation. The study lasted for 3 months from the 1st week of March 2021 to the 1st week of June 2021 (Figure 3).

The inclusion criteria of the houses in study areas included having a water reservoir with a diameter of less than 120 cm, the house owner being willing to drain the water container to clean the previous investment of eggs and larvae before the research begins, willing to use the nets for their water reservoir, willing to comply with all instructions regarding the intervention, such as reporting if the nets torn or damaged and immediately closing the mosquito net after took the water. The water containers for all treatments were always filled with water, draining the container was prohibited, and there was no larva-eating fish dwell in the container to prevent bias. As for the exclusion criteria, all conditions conflict with the inclusion criteria.

This study involved 150 houses and 672 water-holding containers found in all housing environments in the study areas and intervened 116 among them. Most of the water reservoirs are indoors such as bathtubs, buckets, and refrigerator reservoirs. Meanwhile, the outdoor shelters were in the form of drums and used goods. From the number of water reservoirs, it can be divided based on the type as described in Table 1.

The parties who participated in this study were the community in the selected RT, cadres as vector surveillance and sanitarian officers at the Graha Indah Community Health Center as field coordinators, and RT heads as protectors and supporters of activities in their area.

Intervention

All activities in this study followed the health protocol for preventing the transmission of COVID-19 from the Indonesian Ministry of Health. Before commencing the study work, the cadres who helped the researchers were given some directions.

The intervention begins by explaining to the community in the net clusters what will be applied to the water reservoir in their home for the next 3 months from early March 2021 to early June 2021 and its objectives; then, they signed the informed consent. The larval examination and the calculation of the entomological indices were carried out before the intervention; then, the community was given 7 days to drain the water in the containers that had been selected for intervention. After the reservoirs were filled with new water, the nets were installed in water reservoirs and tightened. The community was given instructions to close the net back after they took water and report immediately if the net was damaged.

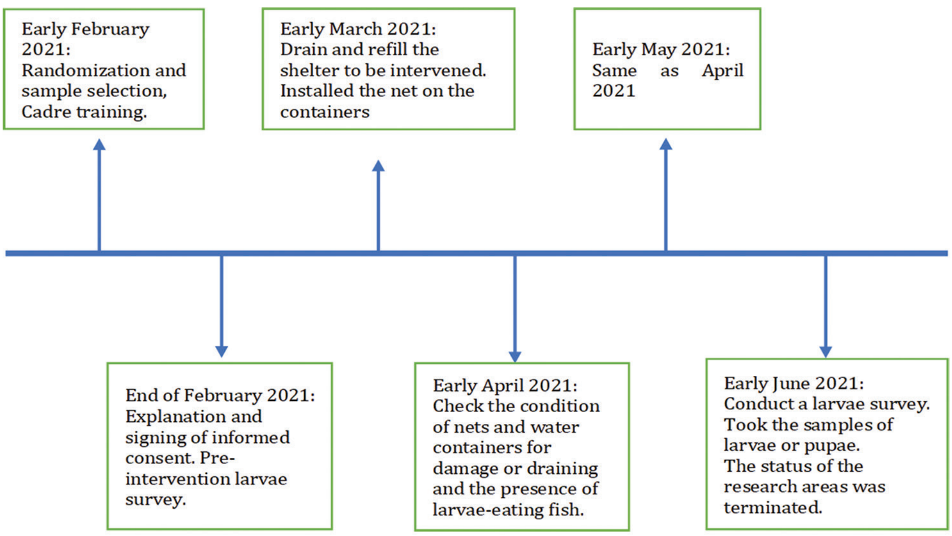

Figure 3: Research Timeline

Table 1: Type and quantity of the water-holding containers in study areas and control

Type of water-holding containers	Net			Control		
	Net 1, n (%)	Net 2, n (%)	Net 3, n (%)	Control 1, n (%)	Control 2, n (%)	Control 3, n (%)
Drums	49 (45.4)	44 (32)	55 (50.0)	35 (34.3)	39 (41.5)	51 (42.9)
Water tank/water reservoirs	8 (7.4)	17 (12)	6 (5.5)	9 (8.8)	12 (12.8)	11 (9.2)
Bathroom tubs	41 (38.0)	32 (23)	10 (9.1)	27 (26.5)	19 (20.2)	25 (21.0)
Barrels/crocks	0	0	17 (15.5)	9 (8.8)	3 (3.2)	9 (7.6)
Plastic buckets	1 (0.9)	27 (19)	9 (8.2)	5 (4.9)	0 (	2 (1.7)
Refrigerator reservoir	8 (7.4)	9 (6)	11 (10.0)	9 (8.8)	12 (12.8)	8 (6.7)
Water dispenser reservoir	0	1 (1)	0	2 (2.0)	3 (3.2)	3 (2.5)
Used cans	0	2 (1)	1 (0.9)	3 (2.9)	2 (2.1)	3 (2.5)
Used plastic containers	0	4 (3)	0	2 (2.0)	2 (2.1)	4 (3.4)
Tires	0	1 (1)	0	1 (1.0)	0	1 (0.8)
Flower vase	1 (0.9)	1 (1)	1 (0.9)	0	1 (1.1)	1 (0.8)
Animal feed-bowl	0	0	0	0	1 (1.1)	1 (0.8)
Others	0	1 (1)	0	0	0	0
Total	108 (100)	139 (100)	110 (100)	102 (100)	94 (100)	119 (100)

The intervention was maintained for 3 months, and every month the cadres checked whether there were damaged nets, any larva-eating fish found in intervened containers, and have the containers were drained. House owners can request new nets at any time if their nets were damaged during the intervention period.

After 3 months of treatment, larval surveys were conducted in all water reservoirs in the home environment. The larvae and pupae found in intervention clusters were taken as samples to identify the genus. The immature mosquitos were put into transparent containers with lid along with water from their natural habitat, labeled with the name of the head of the household where the larvae and pupae were found, RT, and house number. Larvae were identified based on simple physical characteristics that can be seen with a magnification of 10 times using a magnifying glass such as the appearance of the thorax and abdomen, siphon shape, hair distribution and morphology, as well as the movement and position of pupae and larvae in the water [15], [16], [17]. The larvae that have been identified were put back into the pot until it changed into adult mosquitos and re-identification was conducted based on physical appearance with a magnification of 10 times. The difference in the identification results between larvae or pupae and adult mosquitoes took the identification results of adult mosquitoes as the final result.

Ethics

This research has been approved by the Health Research Ethics Commission, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia, number: 2068/UUN4.14.1/TP/02.02/2021.

Results

The primary data obtained from observations during the study were the number of water reservoirs, residents' water sources, entomological indices, and genus of mosquito larvae found in the study area. The secondary data were obtained from the Balikpapan Health Office, Graha Indah Community Health Center officers, the Balikpapan City Government's website, and the Balikpapan's annual report in the form of data on the number of houses and data on dengue cases.

The results were assessed using a larval density survey, conducted before and after the treatment/intervention, including several indicators, e.g., Free Larva Index (FLI: percentage of houses without larvae), house index (HI: percentage of houses with larvae/pupae), container index (CI: percentage of water reservoirs filled with larvae or pupae), and BI (number of positive containers per 100 houses inspected) (Figure 4). In the dengue hemorrhagic fever control program in Indonesia, the FLI target is set ≥95% to reduce the transmission of DHF [18], [19].

Research samples were 6 clusters from 6 RTs in Graha Indah subdistrict, Balikpapan city, Indonesia, with a total of 150 houses, 672 water reservoirs, and 116 intervened containers; none was eliminated during the study which lasted from the 1st week of March 2021 to the 1st week of June 2021.

The majority of residents have drums made of plastic or metal, both as indoor containers for consumption (tightly lid containers) or outdoors containers to collect rainwater (not covered) and other non-consumable water containers (closed but not tightly closed). The bathroom tubs were on average the second largest water container in the research area in general. As many as 11.5% of the shelters in the three research areas are small shelters that are prone to be neglected and have the potential as breeding places, such as refrigerator reservoirs, used cans, and flower vases (Table 1).

The majority of the water sources used were from waterwork municipal, managed by the local government of Balikpapan. Meanwhile, gallon/bottled water is used for drinking and rainwater for non-consumption use such as cleaning and washing

Table 2: Source of clean water in study areas

Clean water sources	Net			Control		
	Net 1	Net 2	Net 3	Control 1	Control 2	Control 3
MWW + gallon/ bottled water	23 (92)	15 (60)	3 (12)	20 (80)	16 (64)	22 (88)
MWW + rainwater	0	0	0	0	0	0
MWW + rainwater + gallon/bottled water	2 (8)	9 (36)	22 (88)	5 (20)	9 (36)	3 (12)
Rainwater + gallon/ bottled water	0	1 (4)	0	0	0	0
Total	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)

MWW: Waterwork municipal.

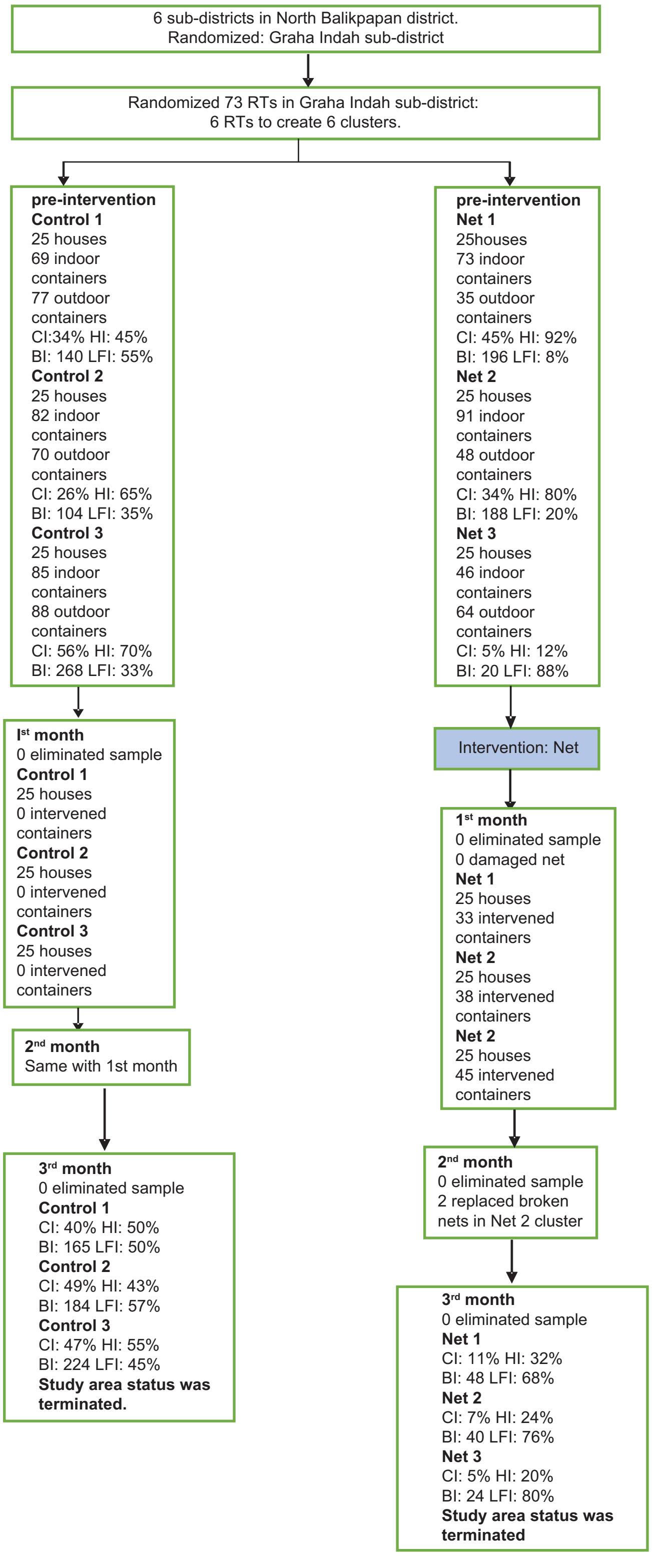

Figure 4: Trial profile, analysis samples, and outcomes

(Table 2). If the water distribution from the waterwork is smooth, the rainwater is not used.

As many as 116 water containers in three clusters were intervened with nets. After 3-month intervention, the net installation was negatively associated with the increase of CI in the intervened containers around 17%-84%. In cluster control, the CI in one cluster experienced increased almost 30%.

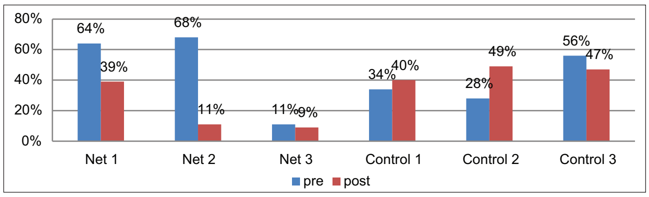

Figure 5: The Container Index in intervened large water-holding containers compared to control group pre-and post-net intervention and in clusters control

However, in the other 2 cluster controls, the CI had minuscules decreased (Figure 5).

On the other hand, after 3 months of intervention with nets towards the large water-holding containers in the study area, the entomological indices of the environment around it, in 2 over 3 study clusters experienced a decrease, especially BI (Figure 6). Meanwhile, the indicator of entomological indices in group control experienced a minor decrease, while the LFI had a diminutive increase (Figure 6).

The bivariate analysis applied paired *t*-test with α = 0.05 showed that *P* value in every entomological index's variable > 0.05 (Figure 7). It concludes that net intervention did not affect the entomological indices statistically, although empirically it showed differences in results between pre- and post-intervention.

Following the post-intervention survey, we took samples of larvae found in water containers in net clusters. Although it was not related to the study, we at least obtained a general depiction of the genus of the mosquitoes that developed and thrive in water reservoirs in the intervention areas (Figure 7).

The results of the identification of immature and mature mosquitoes revealed that 46% of the larvae found in water reservoirs were *Aedes*, and the rest 54% were *Culex*. We were unable to go further into detail about the species because we only observed the macro-physical characteristics using 40x magnification, for an instance; the larval behavior in the water, the siphone (air tube), the palmate hair, and the comb scale. Also, the head, the torso, the legs, and the color of mature mosquitos [13], [14], [15]. Identification was conducted manually because the entomological laboratory facility unavailable in Balikpapan, and had difficulty to reach entomological laboratories in another city due to limited access and social distancing measures during COVID-19 case increase in May-August 2021.

Discussion

To our knowledge, this is the first report of a quasi-experimental study with pre-test and post-test measurements related to the use of non-insecticidal nets in vector control of dengue hemorrhagic fever in Indonesia.

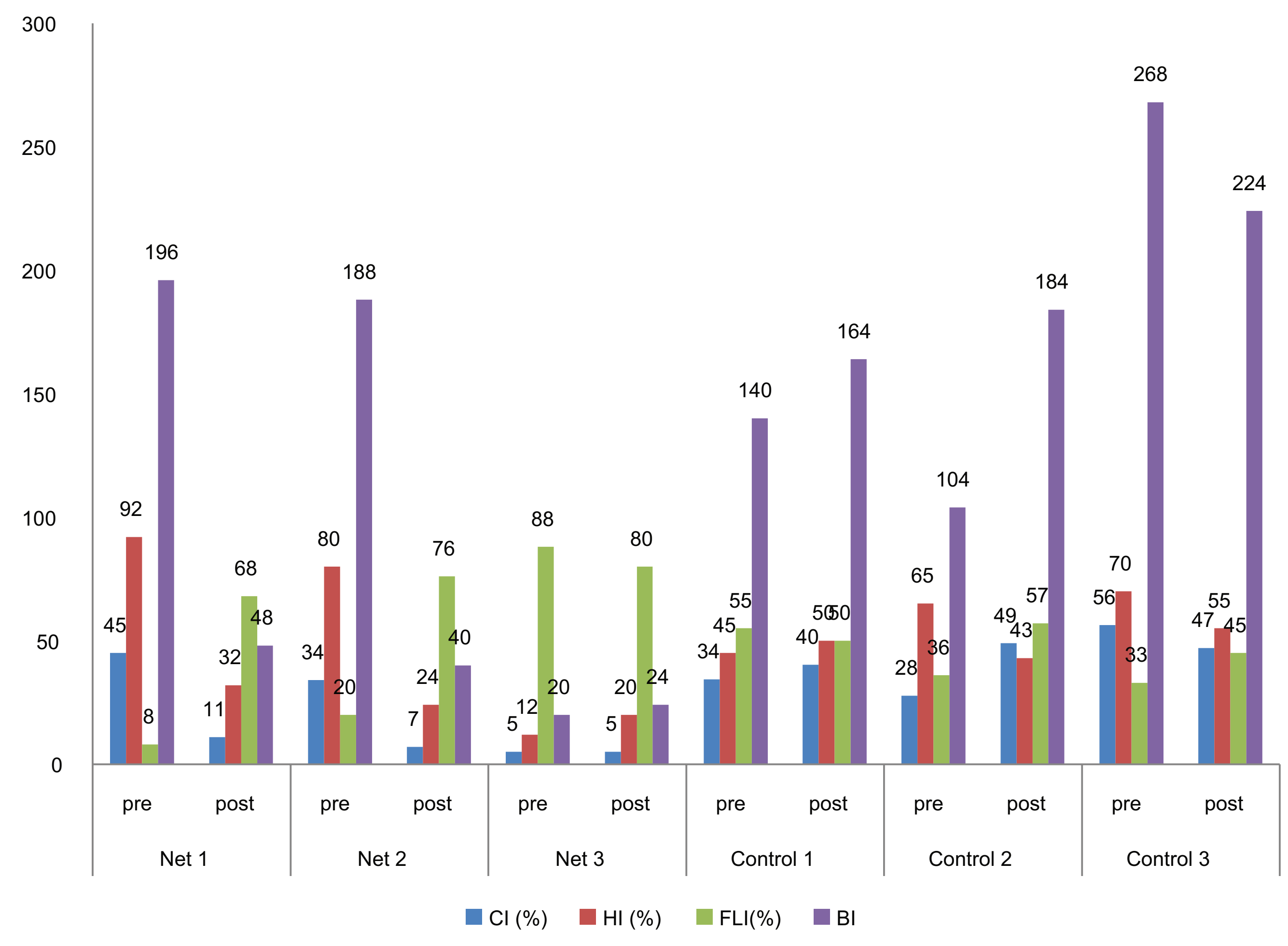

Figure 6: The environment entomological indices pre-and post-net intervention and in control clusters. CI: Container index (%); HI: House index (%); BI: Breteau index; LFI: Larvae-Free Index (%)

Various types of containers can be breeding grounds for immature mosquitoes, but certain shelters can produce more and efficient more for developing immature mosquitoes [20]. The most prominent habitat types for vector breeding were buckets, drums, tires, and pots which produced 75% of pupae from all breeding sites [21]. However, the open larger container played a significant role as vector factories in the environment. The wide-open the mouth of the container and the larger the size, the greater number of immature mosquitoes will be produced [7]. Hence, in this study, we targeted the larger water containers to observe if nets treatment manages to reduce the density of immature mosquitos in the water container, so the number of adult mosquitos in nature could deflate.

Tsunoda *et al*. in Vietnam conducted a field trial using insecticide net, Olyset® Net, which revealed that it managed to reduce the density of immature *A. aegypti* in 1 month after installation in all trial areas [22]. Kittapayong and Strickman pioneered the use of net as a water container cover, which revealed that after 7 days of exposure, no mosquito larvae were found in the jars [23].

In this study, the non-insecticide tulle net intervention showed an association with reduction of all entomological indices' panels in 2 out of 3 intervention clusters (Figure 6). CI decreased 75%–79%, HI decreased 65%–70%, and BI decreased 75.5%–78.7%, while FLI rose 73.7%–88%. Meanwhile, the control clusters demonstrated opposite pattern, 2 clusters underwent a significant increase in entomological indices and drop in LFI, while 1 cluster had minuscule escalated in LFI and unsignificant decreased in entomological indices. However, for the intervened containers, nets performed good by reducing the CI.

Table 3: Bivariate analysis with paired t-test net-intervened clusters (n = 3)

Parameters	Mean	SD	SEM	95% CI of the difference		*t*	df	Significant
				Lower	Upper			
Container index	20.333	17.954	10.366	−24.266	64.933	1.962	2	0.189
House index	36.000	38.158	22.030	−58.789	130.789	1.634	2	0.244
Breteau index	97.333	87.757	50.667	−120.668	315.334	1.921	2	0.195
Larva free index	−36.000	38.158	22.030	−130.789	58.789	−1.634	2	0.244

SD: Standard deviation, SEM: Standard error of mean, CI: Confidence interval.

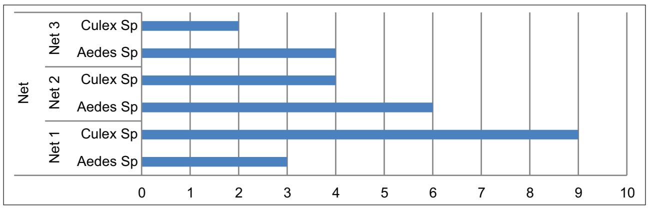

Figure 7: The number of containers and the genus of mosquitos found in the containers.

Referring to bivariate analysis showed that nets did not affect the environment entomological indices. It may conclude that the various results in entomological indices might be influenced by weather and climate since it affected the environment as well as played a significant role in dengue transmission and vector development [24].

Tulle nets, whose physical form and function are the same as mosquito net theoretically able to prevent adult mosquito contact with the water surface, prevent oviposition in water containers, hence reducing the density of immature mosquitoes in water reservoirs. Closing the water containers with nets empirically has impacts on the entomological indices by reducing CI, HI, BI, and FLI since no mosquitoes were able to lay eggs on it. Refer to Islam *et al*., the larger water reservoirs produce more immature mosquitoes, in consequence, closing the larger containers managed to reduce the density of mosquitos' larva and decreased the abundance of adult mosquitoes in the environment. The decline in adult mosquitoes also means a decrease in the number of eggs and production of immature mosquitoes; it created a negative cycle for mosquito which the result was population decline. Hence, the results of the study show that net negatively affects the CI, HI, and BI by lowering them, and positively affects the LFI by increasing it.

Larval control using nets is one of the methods to reduce the source of mosquitoes by changing or eliminating mosquito breeding sites including closing or filtering water containers, and eliminating or changing breeding places so that they are not suitable for larval development [25]. Unlike larvicides that kill larvae, the net is working indirectly toward entomological indices. It is a physical vector control, prevents mosquitoes from contact with water, and prevents adult mosquitoes that developed from escaping the water containers. Net with pore diameter <2–3 mm trapped young mosquito escape from breeding site to find food. Young mosquito cannot survive its early life as a mature more than 4 days due to starving [26] or submerged by water when the water level rises (effective in abandoned rainwater container where the gathered rainwater is left unused for some reasons). Therefore, the entomological index reduction did not occur instantaneously. The net intervention is suitable for long-term containers that are rarely drained because they are too large or neglected, such as rainwater storage, drums outside the house, and water reservoirs that were located high.

However, the use of nets is vulnerable to the compliance of the residents to reinstall the net after they access the water from the mouth of the reservoir. Therefore, if residents are negligent, mosquitoes have the opportunity to enter and lay eggs on the water surface.

In terms of eradicating vector breeding places, in addition to its effectiveness, the net can help the community more easily perform vector control independently because large containers are protected with nets so that the community and cadres can pay more attention on small water-holding containers which are numerous around the house but tend to be neglected. In addition, by using nets on outdoor reservoirs, especially in areas with vegetation, the debris from plants are prevented from contaminating the water.

The majority of residents' water sources came from municipal waterworks. In the rainy season, according to residents, water from waterworks was very smooth distributed in their areas; hence, they left the rainwater unused. The result was an accumulation of rainwater in reservoirs which became potential breeding places for immature mosquitoes even in areas where temephos is used as a larvicide because temephos is susceptible to water turnover [27], [28], [29], [30]. This is why the installation of nets might manage the vector control to overcome the susceptibility of temephos toward high water turnover in the rainy season. However, the effectiveness of its combination needed further study.

Need to be improved in this study is a survey for adult mosquitoes, which made it not possible to reveal the density levels of pre- and post-intervention toward the abundance of adult *Aedes* mosquitoes in that area. The number of houses used for the study was limited; the duration of the study was short due to the COVID-19 pandemic, which made the researchers unable to conclude the effects of 3-month intervention toward the cases of dengue fever in the Graha Indah subdistrict let alone in Balikpapan city. Furthermore, the identification of larvae was conducted manually instead of entomology laboratory examination.

Conclusions

Nets show positive impact by lowering the entomological indices of the water containers and the environment around it. It is not affected by water turnover and can keep the water clean from debris. The effectiveness of the net intervention is influenced by the community's compliance with the correct use of the net for maximum protection. For mass implementation, it is recommended to ensure that the community understands how the net covering the water reservoir works. Net is suitable for areas where temephos is averse or unable to provide it.

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