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Research Article
Key Breeding Place for Dengue Vectors and the Impact of Larvae Density on Dengue Transmission in North Sumatera Province, Indonesia

[1]Fazidah A. Siregar, [2]Tri Makmur and [3]Nurul Huda

[1]Faculty of Public Health, University of Sumatera Utara, 20155 Medan, North Sumatera, Indonesia
[2]Faculty of Medicine, Islamic University of Sumatera Utara, Medan, North Sumatera, Indonesia
[3]School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, UniSZA 22200, Besut Campus, Terengganu Darul Iman, Malaysia

Abstract
Background: The existence and control of Dengue Hemorrhagic Fever (DHF) are closely related to its vectors. Larval density and key breeding sites play important roles in determining the most suitable and effective methods for vector control. **Materials and Methods:** The present study identified the common breeding places for both *Aedes aegypti* and *Aedes albopictus* and examined larva density and the impact on dengue transmission. An entomological survey was carried out in Medan and Langkat in 304 households in both districts. **Result:** *Aedes aegypti* and *A. albopictus* were found in 11 and 12 out of 18 types of water containers, respectively, in both high and low incidence districts. *Aedes aegypti* larvae were found in most indoor bathtub containers. By contrast, *A. albopictus* larvae were found in most outdoor pail containers both in high and low incidence districts. **Conclusion:** The larval indices for *A. aegypti*, which is predominantly found in Medan and for *A. albopictus* larvae, which is predominantly found in Langkat were 33, 12 and 39 and 13, 5 and 18, respectively. The House Index (HI) was a determinant of DHF transmission in North Sumatera province. According to the WHO, the larval density figures of HI, Container Index (CI) and Breteau Index (BI) in Medan and Langkat were 5, 4 and 5 and 3, 1 and 2, respectively. Thus, the density figure of larval indices in Medan was higher than in Langkat (4.7 and 2, respectively). Based on the WHO standards, both districts have HI values higher than 10, which indicates that both high and low DHF incidence districts have a high risk for DHF transmission and the probability of transmission for DHF in Medan was higher than in Langkat.

Key words: Key breeding site, dengue vectors, larva density, dengue transmission, North Sumatera

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Corresponding Author: Fazidah A. Siregar, Faculty of Public Health, University of Sumatera Utara, 20155 Medan, North Sumatera, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Vector-borne diseases are global public health risks. It is estimated that 3.6 billion people live in areas of high risk for dengue infection with 230 million infections worldwide[1]. Approximately 50-100 million cases of dengue fever occur annually with 25,000 deaths[2]. Ever since Dengue Hemorrhagic Fever (DHF) was recognized in North Sumatera in 1975, the disease has become an important health problem in North Sumatera province. The Ministry of Health of Indonesia[3] reported that North Sumatera province ranked third highest for DHF cases with 16 dengue endemic districts. The incidence of the disease has increased annually and remains high. In North Sumatera province, the incidence of DHF increased from 24/100,000 population in 2007 to 56.7/100,000 population in 2012 and 66.2/100,000 population[4] in 2015.

The DHF is a complex problem. Many factors contribute to DHF incidence, including socio-demographic, behavioural, cultural and environmental factors. The environment greatly affects the *Aedes* mosquito breeding sites, especially when there are many containers that serve as breeding places for mosquitoes. Ubiquitous breeding sites, such as tree holes, coconut shells, water jars and unused and discarded tires, can be potential breeding sites for *Aedes* mosquitoes[5].

Moreover, the existence of *Aedes* mosquitoes plays a role in disease transmission. Larval indices, which have been used to quantify vector breeding sites and function as indicators to monitor *Aedes* populations for dengue virus transmission, include the Container Index (CI), House Index (HI) and Breteau Index (BI)[6-8].

Thus far, there is no vaccine or specific medication for dengue hemorrhagic fever and vector control has been the right choice for the prevention and control of dengue hemorrhagic fever. Various efforts related to the eradication of vectors have been conducted in the prevention of dengue, such as mosquito nest eradication (PSN), health education and the use of insecticide fogging and abatisasi, but the results have not been as expected. Many potential breeding grounds for mosquitoes are difficult to monitor, such as tin cans, unused and discarded tires and drums, tree holes and others. Moreover, community removal of mosquito breeding sites is not routinely performed and many containers can be a water reservoir, especially in the rainy season.

In developing an effective strategy for DHF control, one approach is the eradication of vectors, especially larvae and focusing on key breeding containers of primary vectors[9]. The key breeding sites of *Aedes* larvae are affected by various factors, such as topographical areas, faith-based communities[10] and high/low risk DHF areas[11]. A study in the Northeastern region of Thailand found that earthen jars and cement tanks were the key breeding sites in both high and low risk DHF areas and there were no significant differences between the two sites in term of larval density. Knowing the key breeding containers for dengue vectors as well as larval density is essential and might result in more effective dengue prevention efforts.

The DHF is still a major health problem in North Sumatera province. Since, the disease was recognized the incidence of the disease annually remains high. *Aedes* mosquito is the main vector that plays a role in disease transmission. The lack of available vaccines and drugs that might result in effective control strategies is critical and needs to be addressed to break the chain of dengue transmission. Various efforts related to the eradication of vectors have been conducted, but the results have not optimum. On the other hand, monitoring for *Aedes* mosquito that identified the common breeding places and determine larval density have not regularly conducted. Therefore, this study was undertaken to provide basic information in the mapping of breeding places of *Aedes* mosquitoes and to determine the larval density to help health organizations develop effective control strategies for dengue hemorrhagic fever, mainly in North Sumatera province.

MATERIALS AND METHODS

The entomological study was carried out in Medan, a district with high DHF incidence and Langkat, a district with low DHF incidence, in North Sumatera province from 5th April, 2011 to 16th May, 2011. In the present study, only one visit was made to each selected house. A larval survey was conducted based on WHO procedure and involved the examination of both indoor and outdoor water containers to look for the presence of *Aedes* larvae. For each positive container, a larva was pipetted into a plastic cup or plastic bag and brought back to the laboratory for identification. The House Index (HI), Container Index (CI) and Breteau Index (BI) were calculated to determine the larval density.

Sample: The study population was households in both districts with high DHF incidence (HIDs) and low DHF incidence (LIDs). Multistage sampling was used to select the study population. In the first stage, one district with high DHF incidence and one district with low DHF incidence were purposively selected based on the incidence of DHF in the previous year. Medan, a district with high DHF incidence and Langkat, a district with low DHF incidence, in the North Sumatera province were ultimately the two districts selected for the entomological study (Fig. 1). In the second stage, two subdistricts, one with the highest and the other with the

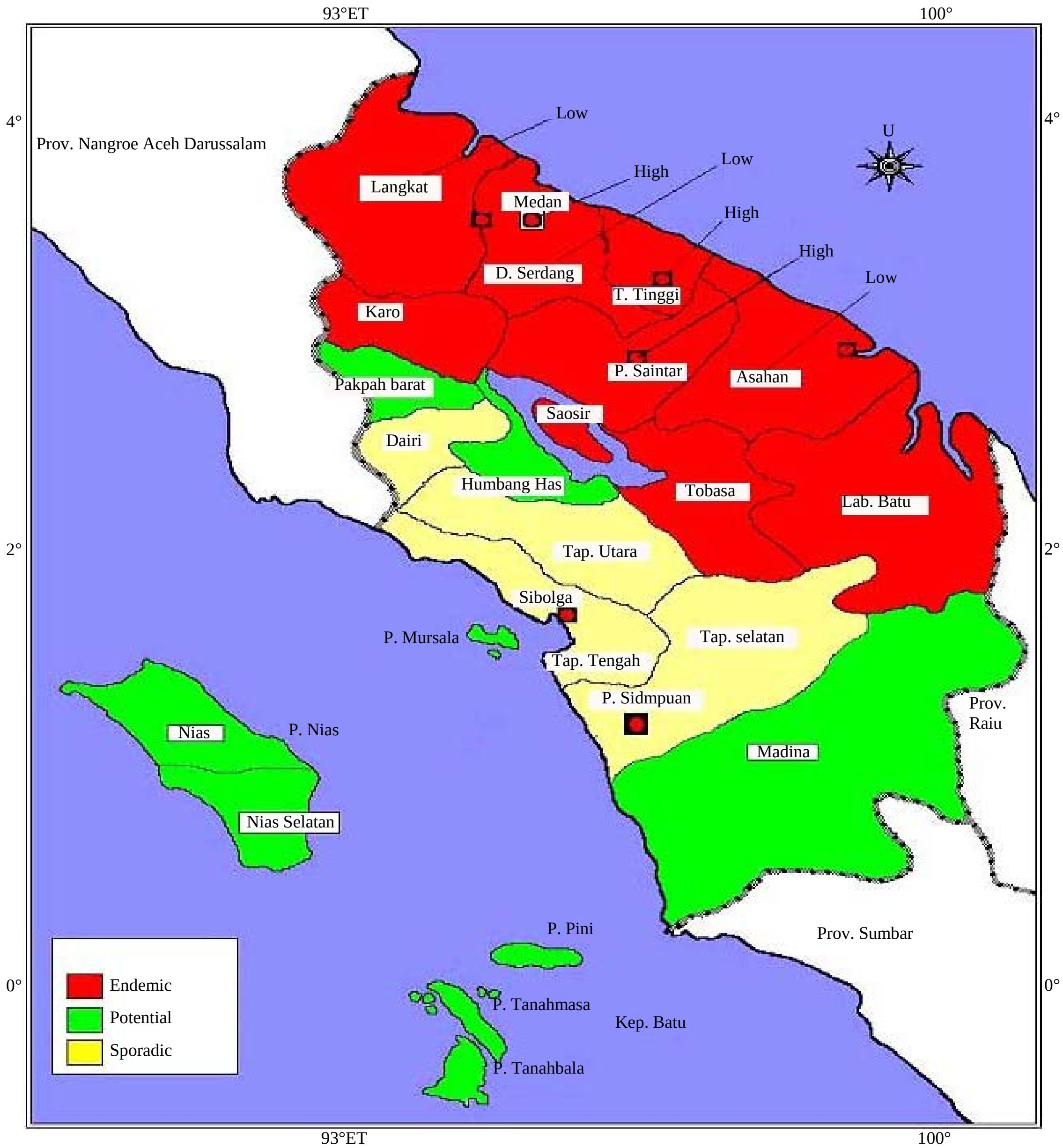

Fig. 1: Map of North Sumatera province

lowest DHF cases were again purposively selected for each district. In the third stage, the same criteria as above were applied to select two villages from the subdistricts. For each village, two sub-villages were randomly selected. In the subdistrict with high DHF cases, households were systematically selected among the households with reported cases from the health office registry, whereas, in the low subdistrict, households were selected using systematic sampling based on the registry of the household from the sub-district administrative office.

Sample size: The sample size for our study was calculated using the two-proportion formula[12] with an average proportion of DHF cases in the three districts with high DHF cases (P_1) of 0.156 and an average proportion of DHF cases in the three districts with low DHF cases (P_0) of 0.017 with an allowable error of 5% and a power of study of 80%. Multistage sampling was applied in this study and the sample size was multiplied by the design effect of 2. Therefore, 304 households for districts with both high and low DHF cases were included in this study.

Study instruments: The socio-demographic data were collected using interviews and recorded using questionnaires. The socio-demographic factors included age, sex, education, occupation and number of family members. The housing density was expressed as the average number of persons per household. The cut-off point was 4 persons per household[13]. The larval survey was conducted to determine larval density. The tools used included a survey form, pipettes, plastic bottles, plastic bags, a specimen vial with stoppers, pens, a label and a flashlight. All of the indoor and outdoor containers at the selected houses were inspected to look for the presence of *Aedes* larvae. From each positive container, a larva was pipetted into a plastic cup or plastic bag and brought back to the laboratory for identification and then the findings were recorded on the survey form. Three larval indices, the House Index (HI), Container Index (CI) and Breteau Index (BI) were calculated. The HI is defined as the percentage of houses positive for *Aedes* larvae. The CI is defined as the percentage of containers positive for *Aedes* larvae. BI is defined as the number of containers positive for *Aedes* larvae per 100 houses examined[6-8].

Statistical analysis: The Statistical Package for Social Science (SPSS) program for data analysis (Release 22.0, SPSS Inc., Chicago, Illinois, USA) was used. All of the variables were checked for normality using the Kolmogorov-Smirnov test. The data were presented either as frequency distributions, percentages or proportions and were summarized using tables and diagrams. The socio-demographic information of the respondents as well as larval *Aedes* indices were calculated and tabulated for descriptive statistics. The effect of the mean number of containers and positive containers on DHF transmission was analyzed using independent t test and two-way ANOVA. Simple and multiple logistic regressions were used to analyze the association between larvae indices and DHF transmission. Initially, the data were analyzed using simple logistic regression to select the significant variables with p-values less than 0.25. Then, the significant variables were included in a multiple logistic regression[14].

RESULTS

The majority of respondents both in HIDs and LIDs had low education were not working and were members of households that had more than 4 family members. However, the mean age of respondents in HIDs was higher than LIDs (Table 1).

Table 1: Socio-demographic characteristics of respondents in high and low incidence districts for DHF in North Sumatera province

Variables	HIDs n (%)	LIDs n (%)
Age (year)[a]	41.7 (10.1)	39.1 (9.5)
Sex		
Female	149 (98.0)	151 (99.3)
Male	3 (2.0)	1 (0.7)
Education		
High	57 (37.5)	47 (30.9)
Low	95 (62.5)	105 (69.1)
Occupation		
Not working	97 (63.8)	107 (70.4)
Working	55 (36.2)	45 (29.6)
Number of family members		
<4 person	25 (16.4)	39 (25.7)
≥4 person	127 (83.6)	113 (74.3)

[a]Mean (SD)

Table 2: Comparison of the mean number of containers and positive containers per house between high and low incidence districts for dengue hemorrhagic fever in North Sumatera province by independent t-test

Variables	HIDs mean (SD)	LIDs mean (SD)	t-statistic (df)	p-value
Number of containers per house	3.4 (1.9)	3.7 (2.1)	-1.436 (302)	0.152
Number of positive containers	0.4 (0.7)	0.3 (0.6)	2.268 (302)	0.024

In low incidence districts, there were a higher number of containers but a lower number of positive containers. The type of container positive for *Aedes* larvae in HIDs was more varied than in LIDs (Fig. 2). The pail was the most common container and had the most positive larvae outside the house both in HIDs and LIDs. Meanwhile, the bathtub was the most common container positive for *Aedes* larvae inside the house both in HIDs and LIDs (Fig. 3). There was no significant difference in the mean number of containers per house between the districts with high and low incidence. However, the mean number of positive containers in the district with high DHF incidence was significantly greater compared to the district with low DHF incidence ($p<0.05$) (Table 2). Furthermore, to determine whether the number of positive containers was associated with DHF transmission, a two-way ANOVA was conducted and the number of positive containers was significantly associated with DHF transmission (Table 3).

Aedes aegypti and *Aedes albopictus* were found in 11 and 12 out of 18 types of water containers in both high and low incidence districts, respectively. *Aedes aegypti* larvae were predominantly found in Medan, whereas, *Aedes albopictus* larvae were predominantly found in Langkat (Fig. 4). The larval indices (HI, CI and BI) for *Aedes aegypti* were higher in Medan than in Langkat (33, 12 and 39 and 11, 2 and 9, respectively), whereas, the larval indices (HI, CI and BI) for *Aedes albopictus* were higher in Langkat than in Medan

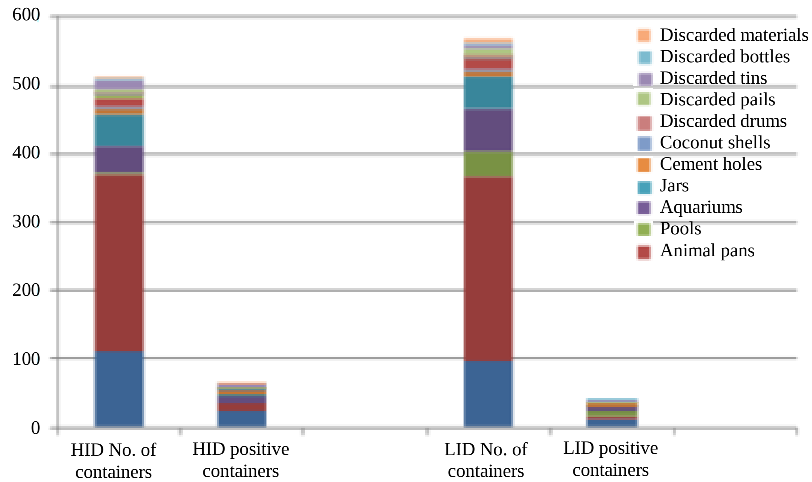

Fig. 2: Types of containers and containers positive for *Aedes* larvae in both high and low incidence districts for DHF in North Sumatera province

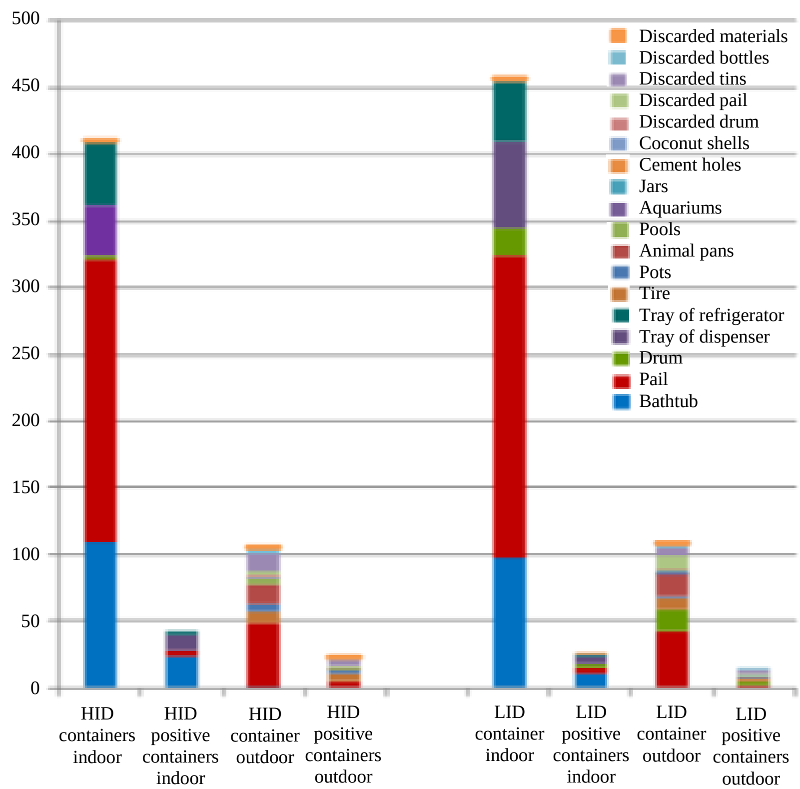

Fig. 3: Types of containers and containers positive for *Aedes* larvae both indoor and outdoor of houses in high and low incidence districts for DHF

Table 3: Effect of the number of containers and positive containers per house on dengue hemorrhagic fever transmission in North Sumatera province using two-way ANOVA

Variables	Adjusted mean (95% CI)	Adjusted mean different (95% CI)	F statistic (df)	p-value
Number of containers per house	1.48 (1.273, 1.696)	0.22 (0.71, 1.15)	0.98 (11, 272)	0.461
Number of positive containers	1.68 (1.55, 1.81)	0.35 (0.10, 0.59)	2.83 (3, 272)	0.039

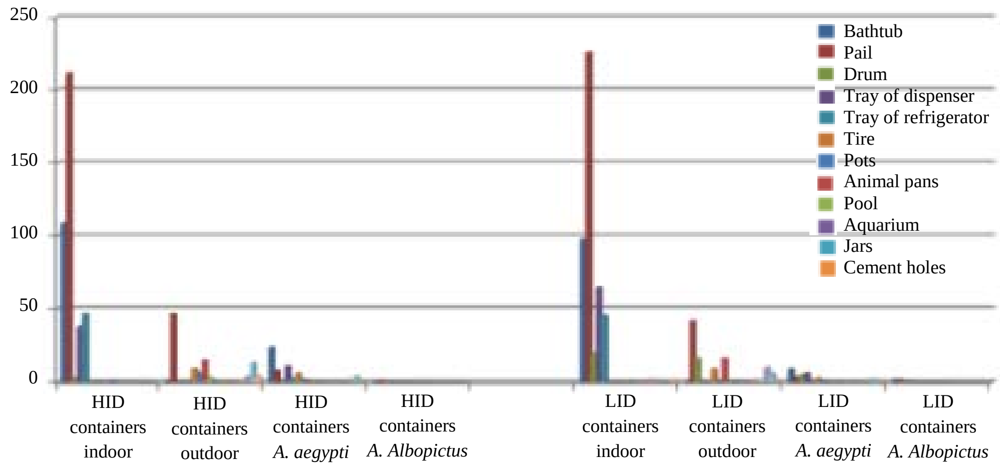

Fig. 4: *Aedes aegypti* and *Aedes albopictus* in indoor and outdoor containers in high and low incidence districts with dengue hemorrhagic fever in North Sumatera province

Table 4: *Aedes* larvae indices in districts with high and low DHF in North Sumatera province

	High incidence district			Low incidence district		
Variables	*Aedes* spp.	*Aedes aegypti*	*Aedes albopictus*	*Aedes* spp.	*Aedes aegypti*	*Aedes albopictus*
Number of households	152	152.0	152.0	152	152.0	152.0
Number of positive households	55	50.0	5.0	33	16.0	19.0
Number of containers	519	519.0	519.0	594	594.0	594.0
Number of positive containers	65	60.0	5.0	45	14.0	28.0
Larval index						
HI	35.0	33.0	3.0	22.0	11.0	13.0
CI	13.0	12.0	1.0	8.0	2.0	5.0
BI	43.0	39.0	3.0	30.0	9.0	18.0

Table 5: Association between larval indices and dengue hemorrhagic fever transmission in North Sumatera province using simple logistic regression

Variables	HIDs n (%)	LIDs n (%)	Crude OR (95% CI)	p-value of wald statistics
House index				
House (+)	54 (35.5)	29 (19.1)	1.093 (3.035, 3.935)	0.001
House (-)	98 (64.5)	123 (80.9)		
Container index				
Container (+)	65 (12.6)	40 (7.0)	1.271 (3.917, 4.125)	0.009
Container (-)	452 (87.4)	527 (93.0)		
Breteau index				
Container (+)	65 (39.9)	40 (24.5)	1.005 (1.622, 6.222)	0.027
Container (-)	98 (60.1)	123 (75.5)		

Table 6: Association between vector indices and dengue hemorrhagic fever transmission in North Sumatera province using multiple logistic regression

Variables	HIDs n (%)	LIDs n (%)	Adjusted OR (95% CI)	p-value
House indices				
House (+)	54 (35.5)	29 (19.1)	1.093 (3.035, 3.935)	0.001
House (-)	98 (64.5)	123 (80.9)		

Forward LR multiple logistic regression was applied. There was no interaction or multicollinearity. Classification table (overall correctly classified percentage = 58.2). An area under the ROC curve (58.2%) was applied to check the model fitness

(13, 5 and 18 and 3, 1 and 3, respectively) (Table 4). Furthermore, the larval indices (HI, CI and BI) were analyzed to determine the factors associated with DHF transmission and the results are presented in Table 5 and 6. This result revealed that House Index (HI) was a determinant of DHF transmission in North Sumatera province. Houses with *Aedes* larvae had 1.093 times odds for DHF transmission compared to houses without larvae. The HI in both high and low DHF incidence districts were higher than the WHO standard for high risk DHF areas (10% HI), which indicated that both districts had a high risk of DHF transmission.

The larval density figures of HI, CI and BI in Medan, a district with high DHF cases and Langkat, a district with low DHF cases were 5, 4 and 5 and 3, 1 and 2, respectively. Based on the density figures of WHO, the density figures for larval indices in Medan and Langkat were 4.7 and 2, respectively, which means that the probability of transmission for DHF was higher in Medan than in Langkat. Moreover, the HI in both high and low DHF incidence districts indicates a high risk of DHF transmission.

DISCUSSION

To combat dengue fever by breaking the chain of disease transmission, it is important to know the breeding places that lead to mosquito eradication, including the types and locations of breeding sites. *Aedes aegypti* and *Aedes albopictus* may have different key breeding places from one area to another[11,15]. Vezzani and Schweigmann[16] found that plastic containers were key breeding sites for *Aedes aegypti* in Buenos Aires, Argentina. Getachew *et al*.[17] found that artificial containers, such as tires, barrels and plastic drums, were the key breeding sites for *Aedes aegypti* in East Ethiopia and most were located outdoor and uncovered. Cruz *et al*.[18] found that drums, used tires and soft drink cans were the major breeding habitats for *Aedes aegypti* in Barangay Poblacion, Philippines. Quintero *et al*.[19] found that tire, pots and cans, were the key breeding sites for *Aedes* aegypti in five urban area of Latin America. Rizzo *et al*.[20] found water tank, drum, washbasin and buckets were the key breeding sites for *Aedes* in Guatemala during the dry season. This study found that indoor bathtubs were key breeding sites for *Aedes aegypti* and outdoor pails were key breeding sites for *Aedes albopictus* in both high and low incidence districts. Our results are contrary to findings from the Promprou *et al*.[11] study in Thailand, which found that the key breeding sites for *Aedes aegypti* and *Aedes albopictus* differed between high and low risk areas.

Aedes aegypti prefer to lay eggs in different containers than *Aedes albopictus*[11]. The mosquitoes lay their eggs in the wall of the water container, where the eggs can survive for several weeks to several months. Phong and Nam[15] studied *Aedes* larvae occurrence in Vietnam and found that *Ae. aegypti* larvae were mostly found in drums, jars, bathroom tanks and concrete tanks, whereas, *A. albopictus* were mainly found in jars, discarded objects, aquarium and drums. Edillo *et al*.[21] in Guba, Cebu city, Philippine found that plastic drums, metal drums and plastic containers were the key breeding sites for *Aedes aegypti*, whereas, bamboo stumps, plastic drums and rubber tires were the key breeding sites for *Aedes albopictus*. Our results revealed that the key breeding sites for *Aedes aegypti* both in high and low incidence districts were bathtubs, pails, drums, trays of dispensers, tray of refrigerators, tires and discarded objects, whereas, the key breeding sites for *Aedes albopictus* were bathtubs, pails and drums. To eliminate the key breeding places for *Aedes* larvae, it was suggested that containers should be regularly emptied.

The presence of containers positive for larvae allows mosquito to breed and increase the mosquito population and risk for dengue transmission. In the present study, there were more containers in LIDs than in HIDs. However, the number of containers positive for *Aedes larvae* was significantly greater in HIDs compared to LIDs. The statistical analysis showed that positive containers were associated with dengue transmission. Phuong *et al*.[22] found that water containers positive for *Aedes larvae* were associated with high incidence of dengue fever in Binh Thuan province, South Vietnam. By contrast, Wongkoon *et al*.[10] found no association between the number of positive containers and DHF risk transmission.

Larval survey was conducted to determine the larval density and monitor distribution and vector abundance. The HI, CI and BI were used to help stratify the DHF risk areas. In this study, *Aedes aegypti* larval indices (HI, CI, BI) were higher in HIDs than in LIDs. On the contrary, *Aedes albopictus* larval indices were higher in LIDs than in HIDs, which might be because *Aedes aegypti* is more common in urban areas (Medan district), whereas, *Aedes albopictus* prefer Brandan (Langkat district), an area that has more vegetation. Larval indices (HI, CI and BI) for *Aedes* larvae in both high and low incidence districts were 35, 13 and 43 and 22, 8 and 34, respectively. A study by Rajendran *et al*.[23] found that the HI, CI and BI in sulurpet India were 9.45, 23.61 and 13.39, respectively. *Aedes aegypti* and *Aedes albopictus* co-existed in the study area. In the Tirunelveli district in Tamil Nadu, India, Bhat and Krishnamoorthy found that the larval indices, similar to house indices, container indices and breteau indices, varied from 5.00-43.3, 0.87-7.50 and 5.00-63.3, respectively[24]. Caprara *et al*.[25] studied the effect of interventions on the vector density and found that social participation and environmental management was significantly reduced the vector density.

The house index was found to be an indicator of DHF transmission in North Sumatera province. Based on the WHO standard for high risk DHF areas (HI 10), it was found that both high and low DHF incidence districts had an HI higher than 10, which indicated a high risk for DHF transmission. A similar finding was shown in the Promprou study in Thailand, which found that both high and low risk DHF areas had a high risk of DHF transmission[11]. Our study revealed that both HIDs and LIDs had HI greater than 10, which means both districts have a high risk for DHF transmission. Furthermore, based on density figures of WHO, Medan has a higher probability for DHF transmission than Langkat.

CONCLUSION

Our study revealed that the key breeding sites for *A. aegypti* and *A. albopictus* did not differ between high and low incidence districts but were more varied for *A. aegypti*. The key breeding sites for *A. aegypti* both in high and low incidence districts were bathtubs, pails, drums, trays of dispensers, trays of refrigerators, tires and discarded objects whereas, the key breeding sites for *A. albopictus* were bathtubs, pails and drums. Moreover, the larval indices (HI, CI and BI) for *A. aegypti* were higher in HIDs than in LIDs and the reverse was true for *A. albopictus*. Both high and low incidence districts had HI greater than 10, which indicated both districts have a high risk of DHF transmission. Effective control strategies for DHF are essential and are focused on vector control. Therefore, a better understanding of key *Aedes* breeding sites and monitoring the larval density are essential to provide information that will help health professionals design more effective prevention and control strategies.

SIGNIFICANCE STATEMENTS

Dengue fever is major health problem in North Sumatra province. Various efforts associated with vector eradication has been done, but the result have not been as expected. Furthermore, monitoring for *Aedes* larval density and the breeding places for *Aedes* mosquito have not been regularly conducted. For an effective control strategy for dengue, is important to know the key breeding site for *Aedes* mosquito and larval density. The main findings of this study reveal that both districts have HI values higher than 10, which indicated that both high and low incidence district had a high risk of DHF transmission. Moreover, bathtub, pails and drums are the common breeding places for *Aedes* mosquito in both districts. These findings lead to the increase of knowledge in the community through health promotion and monitoring activity of vector density are essential for dengue control more effective.

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