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Research article

Effectiveness of space spraying in combating *Aedes aegypti* populations in dengue-endemic areas

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Abstract

Space spraying is a common vector control measure used to suppress *Aedes aegypti* mosquito populations during outbreaks of vector-borne diseases such as dengue. Space spraying can be conducted inside or outside the household or both. The current study assessed the effectiveness of space-spraying in reducing vector populations in an active endemic setting and determined factors associated with indoor spraying acceptability and indoor resting density based on the house density index (HDI) in Songkhla city, Thailand. Between September 2019 and January 2020, adult samples of both sexes of *Ae. aegypti* were collected from 35 households using insect sweep nets and aspirators within sprayed areas in response to ongoing dengue transmission at five time points: day 0 (the day before spraying), and then on days 1, 3, 5 and 7. An oral questionnaire was completed by an adult resident, and the outer premises of the households were inspected for water-holding containers. Attitude toward the effectiveness of indoor spraying was assessed among residents who received both indoor and outdoor spraying as a strategy to prevent dengue (n = 23) and those who only received outdoor spraying (n = 12). Linear regression analysis showed the HDI scores of mosquitoes on days 1, 3 and 5 were significantly lower than the baseline level in households that were sprayed indoors and outdoors. Associated factors for spraying effectiveness included the type of space spraying, the presence of stagnant water near the house and the adoption of preventive practices. The results suggested that the acceptability of indoor spraying was associated with the perception of the local community toward the effectiveness of indoor spraying. Local government campaigns should emphasize the difficulty in controlling *Ae. aegypti* mosquitoes and the need for both government and community action to improve coverage of space spraying and water/waste management during dengue outbreaks.

Introduction

Aedes aegypti is a primary vector of many arboviruses including dengue virus (DENV). Space spraying is a vector control measure recommended by the World Health Organization (WHO) to suppress *Ae. aegypti* adult populations during dengue outbreaks (World Health Organization, 2011). The main purpose of space spraying is to kill the infected mosquitoes thereby interrupting the transmission cycle and stopping the disease cycle. Unfortunately, field data on entomological indices and epidemiological outcomes are rarely reported (Esu et al., 2010). Some studies found a short-lived reduction of *Ae. aegypti*

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populations, as the vector populations returned to pre-spraying level within 7 d after implementation of space spraying (Mani et al., 2005; Koenraadt et al., 2007; Sudsom et al., 2015). Two WHO-supervised trials were conducted in Bangkok, Thailand in 1971 and 1974. The first trial reduced the mosquito population by 99% for 2 wk (Pant et al., 1971), while the second trial maintained reduced levels for 6 mth (Pant et al., 1974). Differences in the reduction period may have been due to the method of fogging, as the latter trial applied fogging from the doorway of the households, while the former was conducted from a roadside vehicle. One study from Putumayo, Columbia showed promising long-term effects of space spraying, with *Ae. aegypti* numbers remaining below pre-spray levels for up to 5 wk (Castro et al., 2007). Evidence of the effectiveness of space spraying for mosquito reduction is still limited and the results from previous studies might not be reproducible in other countries as vector control measures need to be tailored to each setting. In addition, associated factors for the resurgence of this vector were not reported in previous studies. The current study aimed to assess the effectiveness of space spraying in an active endemic setting by assessing the reduction of *Ae. aegypti* populations over time in endemic areas with active dengue cases. Furthermore, factors were identified associated with the reduction of the vector populations.

Materials and Methods

Study area

This study was conducted in Bo Yang sub-district, Muang district of Songkhla province, Thailand. Fig. 1 shows a map of the study site. Bo Yang sub-district is located in the middle of Muang district, in the province capital city of Songkhla, Thailand (100.563°E, 7.204°N; 9.3 km^2) with a human population of approximately 63,000. Muang district is located in the middle of the coastline of Songkhla province (100.35°E, 7.12°N; 189 km^2) with a human population of approximately 165,000. The district has two seasons, a dry season and a rainy season, similar to other provinces in Southern Thailand. The dry season runs from the middle of February until the middle of May, while the rainy season runs from the middle of May until the middle of February (Songkhla Provincial Statistical Office, 2019). Muang district has an average daily temperature of 27.9°C and an average annual precipitation of 2.1 m. The district is one of the top-2 districts with the highest dengue incidence in Songkhla at 162 cases per 100,000 population in 2018 (Communicable Disease Control Department, 2018). Dengue outbreaks generally occur at the beginning and the end of the year. The incidence slowly increases around September to November around the middle of the rainy season, peaking in December and January at the end of rainy season when the temperature is a bit lower. More than 50% of dengue cases in this district consist of children aged 4 to 14 years (Communicable Disease Control Department, 2018). The majority of dwellings in the sub-district are either detached houses or row houses. Most of the detached houses are used for residential purposes while some of the row houses are used for business purposes. Adult-stage mosquito collection, face-to-face interviews with each household representative and environmental observations began in October 2019 and were completed in January 2020.

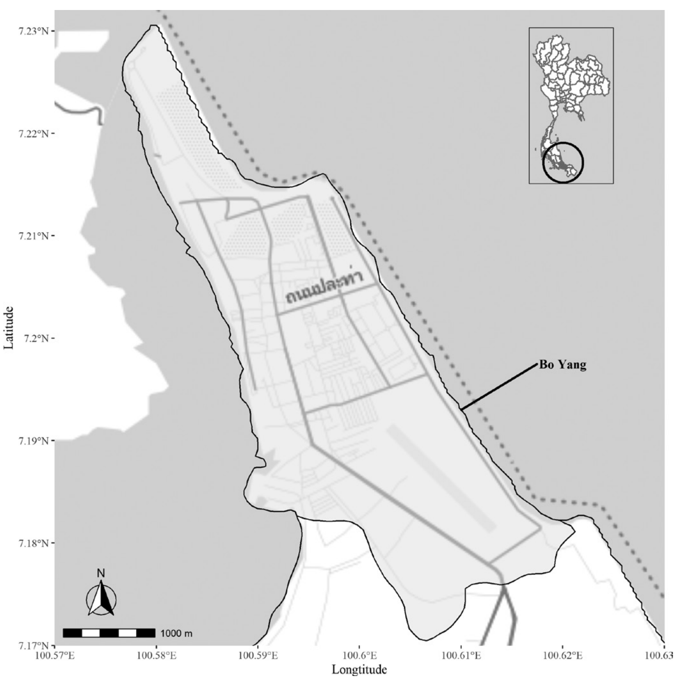

Fig. 1 Study area in Bo Yang sub-district, where circled area in inset map highlights Songkhla province relative to all of Thailand

Study design

The process for data collection is shown in Fig. 2. Mosquito collection was initiated immediately after the researcher received notification of confirmed dengue cases and before the start of spraying by the Local Administrative Office (LAO) took place (day 0) and again on days 1, 3, 5 and 7. The *Ae. aegypti* house density index (HDI) was determined for each household on each collection day by counting the number of resting *Ae. aegypti* mosquitoes collected from both indoors and outdoors (within the perimeter of households) within a standard time period using insect sweep nets and aspirators.

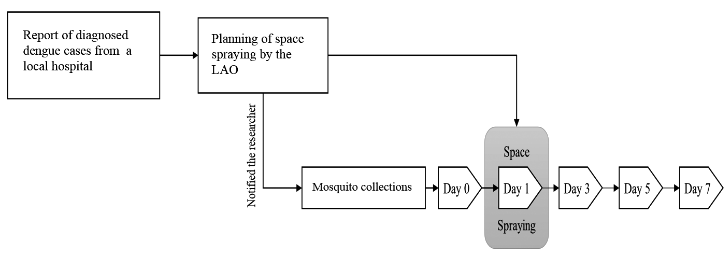

Fig. 2 Flow diagram of mosquito collection process

Sample size

The minimally required sample size of households was computed to compare the HDI scores for *Ae. aegypti* before and after spraying using the two dependent means sample size formula in Equation 1 (Rosner, 2000):

$$n = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \sigma^2}{\Delta^2} \quad (1)$$

where n is the minimum sample size required to detect a difference (Δ) in mean house density index (HDI), σ is the standard deviation of HDI and Z1-α/2 and Z1-β and are standard normal deviates corresponding to the type I and type II error rates. The power (1-β) was set at 80% and the type I error (α) was set at 5%. From a preliminary study involving eight households, the pre-spraying HDI of *Ae. aegypti* was 18.5 with an SD of 10.3. It was hypothesized that the expected HDI of *Ae. aegypti* post-spraying would be reduced to at least half of the pre-spraying level at 9.25 with the same standard deviation based on the previous study conducted in similar areas (Sudsom et al., 2015). Therefore, the expected difference (Δ) in HDI between pre- and post-spraying was 9.25. Based on these established parameters, the required sample size was 10 households.

Data collection

Adult mosquito samples were collected from households of index cases and randomly selected available households in the target spraying areas. Due to time constraints, a maximum of eight houses per target area were selected, including the house of the index case. Each house was revisited five times for mosquito collection. The five target areas with eight households per area should have meant sampling 40 households; however, due to refusals to participate by some households, only 35 households participated in the collection. All samples were collected between 0900 hours and 1600 hours and the average collection time for each household was 30 min. Adult mosquitoes were collected from each household by trained staff using insect sweep nets and mouth-aspirators. Mosquitoes were collected from inside the household and outside within a narrow perimeter (1–3 m). Indoor collection areas included hanging clothes, gaps between furniture, window curtains and all dark areas inside the household such as under the staircase and stove. Mosquitoes were also collected from nearby garage areas, outdoor shoe racks and from outside areas that contained an open-lidded well or water container. All collected mosquitoes from each household were stored and transported in a storage cup with a mesh cover and labelled with the house identification number (house ID) and the day of collection. *Ae. aegypti* mosquitoes were identified from the initial pools using morphological keys for identification by a specialized entomologist (Rueda, 2004).

The clinical history of each dengue case was obtained from the local hospital records. Spraying-related factors included the number of days that had elapsed since the initial onset of dengue fever symptoms, the number of nearby households sprayed, coverage of indoor spraying (percentage of outdoor sprayed households that also received indoor spraying) and the spraying distance (the distance of space spraying applied in the neighborhood surrounding the index cases). This information was collected on the day of spraying (day 1) using a field datasheet. Household-level variables included dengue-related household information such as history of previous dengue infection among household members and whether a household had received any space spraying in the past year, environmental information, and the household members' acceptability and attitude toward space spraying. Information was collected using face-to-face interviews with a structured questionnaire on the day before spraying. Environmental information for each household such as the presence of stagnant water, garbage management and housing material were obtained by direct observation.

Existing mosquito control measures run by Local Administrative Office

Space spraying is routinely conducted by the LAO after they receive notification from the local public hospital. The LAO uses two types of space spraying machines: fontan® Portastar S, an ultra-low volume (ULV) cold fogging machine (Swingtec, 2020a) and a high-performance swingfog® SN 50 thermal fog generator (Swingtec, 2020b). The decision on which type to use is based on the size of the target area; cold fogging is normally used when the target area contains an apartment complex, while the thermal fog generator is used when the majority of the target area contains houses. The spraying is usually conducted within the whole block, covering the areas surrounding the index case household. In this study, a swingfog® SN 50 thermal fog generator was used for indoor and outdoor fogging in all households. Deltacide, a mixture of pyrethroids (deltamethrin 0.5% weight per volume (w/v), S-bio-allethrin 0.75% w/v and piperonyl butoxide 10% w/v), was used as the insecticide with a dilution ratio of 1:79, as recommended by the manufacturer for spraying an area of 1,000 m^2. Bioassays conducted by independent laboratory technicians using the same fogging machine and insecticide showed the susceptible status of *Ae. aegypti* with 98.8% mortality rate based on WHO criteria (World Health Organization, 2016).

Statistical analysis

All data recorded in the structured questionnaire and datasheet were transferred to the computer using EpiData version 3.11 (Christiansen and Lauritsen, 2010), validated and corrected for any inconsistencies and then imported into the R software package version 3.6.1 for analysis (R Core Team, 2019). Descriptive statistics were used to summarize all variables using the frequency and percentage for categorical variables and the mean and SD for continuous variables. The main outcome variable was the HDI of *Ae. aegypti*. Longitudinal mean and SD values of the HDI were summarized in a timeline graph. Since the outcome was repeatedly measured over time, a linear regression model was fitted using generalized estimating equations (GEE), with house ID as the unit of analysis, to assess the longitudinal changes in the HDI adjusting for potential confounding factors such as house size, housing material, roofing material and the area that received spraying. The model was also used to determine factors associated with the changes in the HDI over time. The level of significance in this study was set at 0.05 and 95% confidence intervals were calculated for all estimated parameters.

Ethics statements

Human/Animal care or biosafety and all experimental procedures were approved by the Institutional Ethics Committee of the Faculty of Medicine (number: REC.62-200-18-1) and Animal Ethics Committee of the Faculty of Science (registration number: 2562-10-035), Prince of Songkla University, Thailand. Informed consent was obtained from all the representatives of the selected households. Information sheets were given to all selected participants and all participants provided informed consent to participate in the study. Interviews were conducted after permission had been given by the household representatives on each day.

Results and Discussion

Data on insecticide spraying for houses with dengue cases and surrounding households

The demographic and clinical data provided by the LAO revealed five index cases over the period of 4 mth, of which three were diagnosed with dengue fever and two with dengue hemorrhagic fever. Four cases were elementary school or middle school students and one case was aged more than 60 yr. A descriptive summary of space spraying for the five outbreaks is shown in Table 1. All five index case households were fogged indoors and outdoors with a swingfog® SN 50 thermal generator.

Table 1 Characteristics of space spraying conducted in response to active dengue cases

Characteristic	Mean	SD	Range
Spraying distance (m)	212.0	127.4	70–390
Duration of spraying (min)	19.4	4.4	15–25
Elapsed time since symptoms onset (d)	8.8	4.2	6–16
Number of nearby households sprayed	47.2	27.7	20–91
Coverage of indoor spraying (%)	24.0	12.8	9.9–45

Data from face-to-face interviews and environmental observation

Characteristics of the selected households in the study area are shown in Table 2. In total, 35 households were included in the study, of which three belonged to an index case. Two index cases refused to participate in the study. Twenty-five (71%) households perceived mosquitoes as a problem and 24 (68%) stated mosquitoes were commonly found in the living room. However, the pre-spray HDI of *Ae. aegypti* between households that had a perceived problem was not significantly different from those that did not. About a quarter of the households had at least one family member with a previous history of dengue. All households that had a history of dengue agreed to have indoor and outdoor spraying; however, only 54% of households with no dengue history agreed to have both indoor and outdoor spraying. From the face-to-face interviews, a high percentage (83%) of the households had a history of receiving at least one type of space spraying in the past year, and all but two households (94.3%) had applied a vector control method; a popular method was a commercial insect spray such as Baygon® (51.5% of the households). Most (65.7%) households received both types of spraying (indoors and outdoors) while 12 households (34.3%) received outdoor spraying alone. Households that did not agree that indoor spraying improved effectiveness were more likely to receive only the outdoor spraying ($p < 0.001$). The most common type of household was a row house (60%). The majority (97.1%) were built on concrete; however, the material used for roofing varied. Most of the households (85.7%) were surrounded by pockets of waterlogging or contained water-holding containers conducive to *Ae. aegypti* breeding. The containers that were deemed suitable as a breeding habitat included jars or plastic buckets with stagnant water located around the outside the households or inside the households.

Effect of space spraying on Ae. aegypti HDI

In total, 2,477 adult *Ae. aegypti* mosquitoes were collected consisting of 1,280 (51.7%) adult females and 1,197 (48.3%) adult males from 35 households with 7 d of collection in each household; however, two households refused mosquito collection on day 7. Fig. 3 shows the longitudinal changes in the HDI of *Ae. aegypti*. The HDI in indoor and outdoor spraying groups dropped notably on day 1 and slowly increased through days 3 and 5. The HDI of households with stagnant water nearby was higher on all collection days after space spraying was implemented. The results from the GEE analysis (Table 3) supported the result in Fig. 3 that the HDI of *Ae. aegypti* was significantly lower on days 1, 3 and 5 compared to the reference level on day 0 but there was no difference after 7 d.

In households that received indoor and outdoor spraying, the HDI scores decreased on days 1, 3, and 5 by 15.9, 5.6 and 5.1 mosquitoes per house, respectively. The average HDI scores on days 0, 1, 3 and 5 of indoor and outdoor sprayed households were 18.8, 2.9, 13.2 and 13.7, respectively. Households that received only outdoor spraying showed no significant differences in the HDI between days 1, 3, 5 and 7 compared to day 0. There were no significant differences between indoor and outdoor spraying and outdoor only spraying on all collection days except on day 1 where the HDI from households with both types of spraying was highly significantly lower than among households that received outdoor only spraying ($p = 0.001$). Households with no waterlogging or those without water-holding containers present had a highly significantly lower HDI both before and after spraying ($p < 0.001$). In addition, adoption of preventive practices was associated with lower HDI through the course of spraying ($p < 0.001$).

Table 2 Characteristics of selected households in study area

Characteristic	Frequency	
	n	(%)
Vector characteristics		
Mosquito problem within the house		
Yes	25	71.4
No	10	28.6
Area with most mosquitoes		
Living room	17	68.0
Restroom	3	12.0
Outside	3	12.0
Bedroom	2	8.0
History of a family member with dengue		
Yes	9	25.7
No	26	74.3
Prevention practices		
Received at least one type of spraying in past year		
Yes	29	82.9
No	6	17.1
Applied at least one type of vector control method in household		
Yes	33	94.3
No	2	5.7
Applied spatial repellent (coils)		
Yes	12	36.4
No	21	63.6
Applied electronic zapper		
Yes	11	33.3
No	22	66.7
Applied commercial insect spray		
Yes	17	51.5
No	16	48.5
Usage of netting		
In a bedroom	22	62.9
Whole house	3	8.6
None	10	28.6
Type of spraying received		
Indoor and outdoor	23	65.7
Outdoor only	12	34.3
Attitude: indoor spraying can improve effectiveness		
Agree	28	80.0
Not sure/ don't agree	7	20.0
Attitude: Space spraying can control the spreading of dengue		
Agree	25	71.4
Not sure/ don't agree	10	28.6
House characteristics		
Type of dwelling		
Row house	21	60.0
Detached house	7	20.0
Townhouse	7	20.0
Roofing material		
Concrete	13	37.1
Iron sheet	12	34.3
Wood	10	28.6
Housing material		
Concrete	34	97.1
Wood	1	2.9
Presence of a water storage container with closed lid		
Yes	13	41.9
No	18	58.1
Presence of waterlogging or a water container in household		
Yes	30	85.7
No	5	14.3

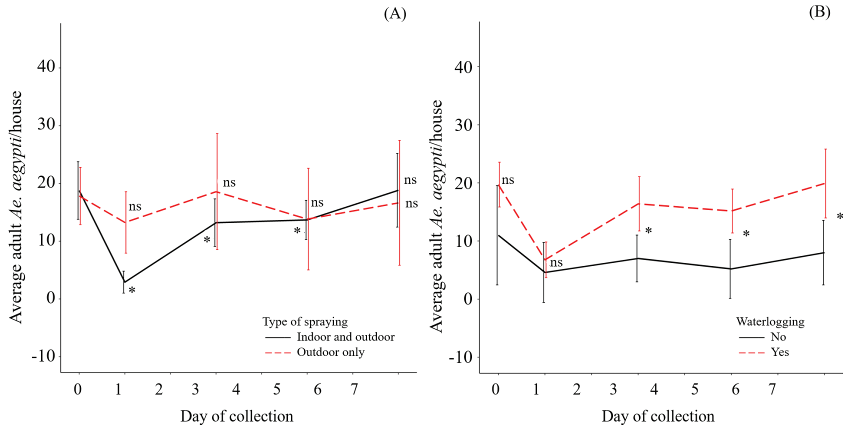

Fig. 3 Longitudinal changes in *Aedes aegypti* house density index (HDI) after implementation of space spraying: (A) stratified by type of spraying; (B) stratified by presence of waterlogging, where * indicates significant differences in HDI compared to baseline levels on day 0 in (A) and significant differences between groups in (B) and error bars indicate the 95% CI of the average HDI.

Table 3 Regression coefficients of space spraying stratified by type: indoor and outdoor versus outdoor only

Variable	Indoor and outdoor				Outdoor only			
	β	Lower CI	Upper CI	*p*-value	β	Lower CI	Upper CI	*p*-value
Days since spraying								
1	-15.87	-20.16	-11.58	< 0.001	-4.58	-10.05	0.88	0.10
3	-5.57	-9.22	-1.91	0.003	0.75	-7.48	8.98	0.86
5	-5.09	-9.96	-0.22	0.04	-4.00	-11.69	3.69	0.31
7	0.03	-5.69	5.74	0.99	-0.91	-8.83	7.02	0.82
Waterlogging (reference = Yes)								
No	-5.12	-9.09	-1.14	0.01	-14.09	-21.03	-7.15	< 0.001
Adoption of prevention practices (reference = Don't apply)								
Apply	-14.08	-17.17	-10.99	< 0.001	-3.8	-5.46	-2.14	< 0.001

CI = confidence interval

The WHO recommends that vector control measures such as space spraying be planned and conducted prior to predicted transmission periods to prevent the further spread of cases (World Health Organization, 2006). However, in practice, space spraying is usually used as a reactive control measure when the transmission period, the febrile phase, has already lapsed. In the current study, responsive space spraying was conducted at an average of 8.8 d (range 6–16) after the onset of symptoms in the index cases. Although the extrinsic incubation period (EIP) generally requires 8–12 d for the viral load to be high enough for transmission (World Health Organization, 2009), a study showed that a high extrinsic incubation temperature could shorten the EIP (Rohani et al., 2009). As the EIP can vary between 2.4 d and 15 d in high-temperature regions (Chan and Johansson, 2012), a faster implementation of space spraying would be more effective in preventing secondary dengue cases following the initial index case.

Regarding the effectiveness of space spraying in this study, implementing indoor space spraying in response to active dengue cases could temporarily reduce the HDI of *Ae. aegypti* mosquitoes for up to 5 d; however, outdoor spraying produced no reduction in the number of *Ae. aegypti* mosquitoes. In addition, the mosquito numbers surged back to baseline levels within 7 d after indoor space spraying was implemented, a pattern similar to that found by Sudsom et al. (2015). However, the differences in the average HDI scores of both male and female adult *Ae. aegypti* in the current study were larger on all collection days compared to Sudsom et al. (2015) that was conducted in the same district. It was hypothesized that this might have been due to the characteristics of the study area with ongoing transmission or without any active cases present as in the study by Sudsom et al. (2015). Comparing the abundance of the mosquito population in areas with ongoing transmission can increase understanding on how space spraying would fare against an actual outbreak, including the role of several factors that come into play such as the coverage and acceptability of space spraying.

The effective duration of indoor space spraying in the current study was short-lived. It was theorized that the rapid increase in *Ae. aegypti* numbers might have been due to the low coverage of indoor spraying (66%) which enabled some *Ae. aegypti* to flee to nearby households where indoor spraying was not conducted. This avoidance behavior could be a response to the repellent effect of the pyrethroid-based insecticide used in the thermal fog generator (Manda et al., 2013). The repellent effect could be a result of a sub-lethal dose of insecticide at the moment of contact. This might have led to a decrease in the resting behavior and an increase in the host-seeking behavior of *Ae. aegypti* in households that did not receive indoor spraying. This phenomenon could potentially lead to further secondary dengue cases. Although 80% of the selected household representatives agreed that indoor spraying would be effective, three of these individuals refused to allow the inside of their household to be sprayed due to inconvenience and the unpleasant odor. A modelling study showed high levels of household coverage (75% treated once per year by indoor residual spraying), applied proactively before the typical dengue season, could reduce symptomatic infections by 89.7% in year 1 and by 78.2% cumulatively over the first 5 yr of an annual program (Hladish et al., 2018). Likewise, lower coverage had correspondingly lower effectiveness, as did reactive campaigns.

Regarding community awareness of mosquito infestation, we found that the pre-sprayed HDI scores of *Ae. aegypti* in households that had no perceived mosquito infestation problem were not significantly lower than households that had this perception. This might have been due to the local residents being accustomed to high numbers of mosquitos in their household as they have lived their whole lives in an endemic area. It is logical for households that have experience with dengue infection to accept and agree to indoor spraying; however, understanding of dengue needs to be translated into action by the whole community to increase the acceptance of indoor and outdoor space spraying. For space spraying to be fully effective in controlling dengue transmission in outbreak areas, integrated vector control programs need to include components that increase public perceptions of the transmission of dengue, awareness of dengue severity and knowledge of the effectiveness of spraying.

The majority (85.7%) of the selected households were located near waterlogged areas or stockpiled water-holding containers. This result corresponded with other studies that showed that water reservoirs commonly found in dengue-endemic areas were attractive breeding habitats for *Ae. aegypti*, especially in areas near or surrounding human habitats due to the feeding behavior of the vector mosquito and its human host preference (Chareonviriyaphap et al., 2003; Nagao et al., 2003; Vijayakumar et al., 2014; Ferede et al., 2018). In contrast to findings from other studies, discarded tires were not found in any of the households in the current study and most waterlogging took the form of blocked gutters, which have been suggested as a possible breeding habitat for *Ae. aegypti* in urban areas (Wongkoon et al., 2013). Furthermore, the water-holding containers consisted mainly of clay jars and neglected wells without a lid. Although different from tires, clay jars, and wells contain the same attributes that make them suitable for *Ae. aegypti* breeding habitats: low temperature, high humidity and a lack of direct exposure to sunlight. The GEE analysis showed that waterlogging and water-holding containers were common, but they also reduced the effectiveness of space spraying. It was hypothesized that this might be due to the mosquitoes seeking a suitable habitat after spraying was implemented, as despite the insecticide initially repelling the *Ae. aegypti*, they returned to the same familiar areas containing stagnant water. Thus, further studies on the resurgence pattern of *Ae. aegypti* in sprayed areas are required. This behavior may be the cause of the resurgence pattern. Similar to several studies conducted in Thailand (Nagao et al., 2003; Thammapalo et al., 2008), a lack of community waste and water drainage management in dengue-endemic areas not only created a habitat for the vector mosquito, but also decreased the effectiveness of the control-measure.

Although information was collected about the perception toward space spraying, including the necessity of indoor spraying and the effectiveness of space spraying in controlling dengue, The current study did not hold any focus group discussions which could provide additional information beneficial for improving the acceptability and coverage of indoor space spraying in the community; quantitative studies on this topic are needed in dengue endemic areas.

In conclusion, rational water and waste management, awareness of dengue severity, and perception of indoor spraying need to be improved for responsive space spraying to be successful in halting dengue outbreaks in endemic areas.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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