ORIGINAL ARTICLE

Awareness and perception of *Wolbachia*-infected *Aedes aegypti* as a dengue control method among residents of Yogyakarta Municipality

Habibi Rohman Rosyad[1], Alan Frederick Geater[1], Citra Indriani[2], Riris Andono Ahmad[3]

[1]Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Thailand
[2]Department of Epidemiology, Biostatistics and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia
[3]Center for Tropical Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia

Corresponding Author: Alan Frederick Geater **Email:** alan.g@psu.ac.th

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ABSTRACT

Dengue is an important communicable disease in Indonesia with an escalating annual incidence. Between 2016 and 2017, a new dengue control method using *Wolbachia*-infected *Aedes aegypti* was implemented in Yogyakarta Municipality. This study aimed to assess community awareness and perception about *Wolbachia*-infected *Ae. aegypti* release as a method of dengue control being implemented in their locality, and to identify the influencing factors. A cross-sectional survey was conducted in 2019 among 484 households in Yogyakarta Municipality. Data were gathered using an electronic data collection tool through face-to-face interviews and direct observations under written informed consent. Weighted estimation and finite population correction were applied in the analysis. Approximately, 23.4% of respondents were aware of the recent *Wolbachia*-based mosquito control program. After being informed about the nature of the control program, 58% of respondents expressed some concern about the possible harmful effects of the release of infected mosquitoes. Concern over possible harmful effects was associated with older age (aOR=1.76, CI=1.04-2.99), a smaller family (aOR=1.86, CI=1.17-2.95), low income (aOR=1.63, CI=1.04-2.57), having an open vegetated area adjacent to the house (aOR=2.02, CI=1.16-3.51), not being aware (aOR=2.26, 95% CI=1.28-3.98) and having low knowledge (aOR=3.00, CI=1.38-6.53) of the *Wolbachia*-based mosquito control program, and perceiving a recent increase in the mosquito population (aOR=2.94, CI=1.43-6.03). In conclusion, awareness of the *Wolbachia*-based dengue control activities was low two years after it was implemented, and low knowledge had a strong association with concern over the possible harmful effects of the release of *Wolbachia*-infected mosquitoes. Long-term community engagement efforts should be made to maintain public awareness and understanding of the *Wolbachia*-based biocontrol method.

Key words:
community perception; dengue control; *Wolbachia*-infected *Aedes aegypti*

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INTRODUCTION

Dengue is the fastest-growing mosquito-borne disease transmitted mainly by the bites of female *Aedes aegypti* mosquitoes.[1] It has become a global epidemic with a 30-fold growth over the last five decades, including an extreme increase in the annual incidence rate in Indonesia, from 0.05 to 77.96 cases per 100,000 person-years.[2,3] Currently, innovative biotechnological interventions e.g., sterile insect technique, genetically modified vectors, and *Wolbachia*-infected *Ae. aegypti*, which could be implemented as population replacement or suppression, have been employed to improve the efficacy of the traditional strategies to prevent and control dengue transmission in Indonesia and other countries.[4-6]

Wolbachia pipientis is a maternally inherited endosymbiotic bacterium that can be found in more than 66% of arthropod species, inducing cytoplasmic incompatibility, an embryonic lethality mechanism that reduces egg hatch and allows *Wolbachia* to spread throughout insect populations.[7-10] Recent studies indicated that *Wolbachia* is found naturally in *Aedes aegypti* mosquitoes at various levels of prevalence although its absence was previously reported.[6,11-16]

Employing *wMel Wolbachia* strain from *Drosophila melanogaster, Wolbachia*-infected *Aedes aegypti* mosquitoes were successfully deployed in diverse settings, reducing local transmission of dengue after its establishment.[17,18] The bacterium decreases the dengue viral load in the ovaries of *Ae. aegypti* mosquitoes, leading to the reduction of dengue infection in *Aedes aegypti* and its vertical transmission among *Wolbachia*-infected mosquitoes.[19]

The implementation of *Wolbachia*-infected mosquitoes varies based on the main objectives of the control method, either to eradicate the mosquito or to spread the bacteria within the mosquito population.

Mosquito population suppression is performed by releasing only male *Wolbachia*-infected mosquitoes, as non-infected females mating with infected males results in reduced egg hatchability. This strategy has been implemented in Thailand, Singapore, and the United States.[20-23] Replacement of the mosquito population with *Wolbachia*-infected mosquitoes is performed by releasing both female and male *Wolbachia*- infected mosquitoes. Eggs from mating male and female mosquitoes that are both infected with *Wolbachia* are also infected. Control of dengue disease results from the reduced transmissibility of the dengue virus in *Wolbachia*-infected females. This is the strategy implemented in Australia, Malaysia, Vietnam, and Indonesia.[18, 24-26]

Reduction in dengue, chikungunya, and zika incidences were reported in previously released areas in Brazil (69%, 56%, and 37% respectively) after three years of its first release.[27] In Australia, *Wolbachia* had been maintained in over 90% of the mosquito population over eight years after completion of release, with an estimated 96% reduction in dengue incidence in treated populations.[25,28] In Yogyakarta, a randomized controlled trial demonstrated a 77% reduction in dengue case incidence and 86% reduction in dengue hospitalizations in *Wolbachia*-treated geographic clusters.[29]

Wolbachia has been reported to be safe for people, other organisms and the environment as it has never been found in fishes, reptiles, birds or mammals.[30] A previous study reported negative PCR results of *Wolbachia* in mosquitoes' saliva although it was present in the salivary glands as the size of the bacterium was greater than the diameter of a mosquito

salivary ducts (around 1-2 μm and less than 1 μm, respectively).[31] Additionally, no adverse consequences were reported by human volunteers who received thousands of bites by *Wolbachia*-infected mosquitoes.[30]

Several studies have been conducted to assess community reaction to vector control employing mosquito releases. A household survey conducted in Vietnam prior to the release of *Wolbachia*-infected mosquitoes showed that releasing *Wolbachia*-infected mosquitoes was acceptable (86%) in the community.[26] Similarly, a public sentiment survey in Singapore reported that the community had no concerns regarding the release of male Wolbachia-infected *Ae. aegypti* mosquitoes in their neighbourhood.[32] However, a community perception survey following the release of genetically modified mosquitoes in Florida for population suppression showed that 51% of respondents raised a concern regarding the mosquito being released.[33]

In Yogyakarta Municipality, within the scope of research, mosquito release containers (MRCs) containing *Wolbachia*-infected *Ae. aegypti* eggs were placed in outdoor areas between 2016 and 2017.[34,35] The purposes of the current study were to assess community awareness of the control program and their perception regarding the release of *Wolbachia*-infected *Ae. aegypti* in their locality, and to identify factors influencing their concern over possible harmful effects. In addition, the level of knowledge about dengue disease, planned action when dengue-suggestive symptoms develop, and changes in preventive behaviors after being aware of implementation of the control program were explored.

MATERIALS AND METHODS

A cross-sectional survey was conducted in June 2019 in Yogyakarta Municipality, Indonesia. The city covers an area of 32.5 square kilometers with a population of 422,732 residents in 2017. Administratively, Yogyakarta City is divided into 14 districts that are subdivided into 45 villages and 2532 neighborhoods called rukun tetangga.[36]

A two-stage cluster sampling was used to recruit 504 participating households from 42 neighborhoods. First, the neighbourhoods were selected randomly using a list of the neighbourhoods in the Municipality. Then, within each selected neighborhood, 12 households were selected using a pre-prepared list of random numbers, matched with the list of households obtained from the leader of each neighborhood. The random sampling to select the neighborhoods and the pre-prepared random numbers for households to be enrolled in the study was performed using R software version 3.6.3.[37] Eligibility criteria included residence in Yogyakarta municipality for at least six months and age ≥18 years. Prior to the data collection, 12 recruited enumerators attended a one-week training consisting of brief information regarding the implementation of *Wolbachia*-infected *Ae. aegypti* in Yogyakarta, the aim of the current study and the data collection procedures. The training included simulation of the interviews and a trial of performing data collection. During the study period, the enumerators conducted interviews with selected participants after obtaining written consent through the informed consent process. Open Data Kit (ODK) was used for data collection.[38] Prior to leaving each participant's house, the enumerator checked for the completeness of the data collection form and directly uploaded it to the server. Two supervisors were assigned to ensure the quality of the data collection process through spot checks and cross checks. A data manager was assigned to perform a

double-check on each data sheet uploaded on a daily basis for further controlling the quality of data.

A structured questionnaire was used to obtain information on sociodemographic characteristics, awareness and perception about the program involving the local release of *Wolbachia*-infected *Aedes aegypti*, changes in their preventive practices because of the program, knowledge about, and previous experience of dengue, and planned action if symptoms suggestive of dengue were to occur. Awareness was assessed using the question "*Have you ever heard of Wolbachia-infected Aedes aegypti?"*, and perception was assessed with the question "*Do you have any concern over possible harmful effects of Wolbachia-infected Aedes aegypti?"*.

Questions on knowledge were designed to elicit unprompted answers regarding the cause and vector of dengue infection, dengue control methods and the signs and symptoms of dengue severity. Knowledge of dengue was examined using five questions. Respondents were scored one for each correct answer, and zero for an incorrect one. To discriminate between respondents with better knowledge and those with poorer knowledge, an arbitrary cut-point was used to categorize the knowledge as high if respondents could answer three or more questions and low if it is <3. Respondents were also asked if they knew that *Wolbachia*-infected mosquitoes were being released in their area; if they were unaware, the researcher team delivered a short explanation regarding the concept and process of the program. Knowledge of *Wolbachia* was examined using three questions: "*What is Wolbachia*?", "*Can Wolbachia be passed on to the next generation of Aedes aegypti*?", and "*Can Ae. aegypti transmit Wolbachia to humans*?". As for knowledge of dengue, an arbitrary cut-point was used. Knowledge of *Wolbachia* was categorized as high when at least two questions were answered correctly and low when only one question or less was answered correctly. Two more questions were designed to further elicit respondents' concern over the *Wolbachia*-based program, namely *"Can Wolbachia-infected Ae. aegypti reduce dengue transmission?"* and *"Do you think Wolbachia will affect your health?"*.

Statistical analyses were performed using R software. Frequency and percentages were calculated for categorical variables, while median and interquartile ranges were calculated for continuous variables. Association between each variable and initial awareness of the control program and concern about possible harmful effects of the release of *Wolbachia*-infected *Ae. aegypti* was first analyzed using univariate analyses. Multivariate logistic regression models based on precompiled directed acyclic graphs (DAG) were then constructed to identify the total effect of variables influencing initial awareness and worry about the harmful effects of the control program, appropriately adjusted to minimize bias.[39] Sample weighting with a finite population correction was performed using the 'survey' package in R software to compensate for unequal probability of being selected.[40] The level of statistical significance was set at <0.05.

The data collection was performed after obtaining ethics permissions from the Human Research Ethics Committee, Faculty of Medicine, Prince of Songkla University, Thailand (REC: 61-384-18-2) and the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University, Indonesia (Ref. No.: KE/FK/0353/EC/2019).

RESULTS

Sociodemographic characteristics of the respondents

Out of 504 households visited, 484 were willing to participate in this study, giving a response rate of 96%. Respondents were mostly Javanese (95.1%), Muslim (86.7%), and married (88.8%). The median age was 52 years (IQR = 43, 60, range 21-82 years). Large proportions of respondents were self-employed (41.6%), had an average monthly income of at least 1,846,400 Rupiahs (equivalent to 120 USD) (67.8%), had finished senior high school or university (69.5%), and owned health insurance (86.1%) provided by either the public or the private sector. Further details of the demographic characteristics are shown in Table 1.

Table 1 Socio-demographic characteristics of participants. (n=484)

Characteristic	Category	Number	Weighted % (95% CI)
Sex	Male	219	45.9 (41.4-50.4)
	Female	265	54.1 (49.6-58.6)
Age (years)	21-40	99	20.7 (17.3-24.6)
	41-60	269	56.4 (52.1-60.6)
	>60	116	22.9 (19.1-27.2)
Education level	Elementary school	65	13.7 (10.1-18.4)
	Junior high school	75	16.8 (13.3-20.9)
	Senior high school	202	40.3 (35.4-45.4)
	University graduate	142	29.2 (24.5-34.4)
Marital status	Single/widowed	58	11.2 (8.2-15.1)
	Married	426	88.8 (84.9-91.8)
Household size	1 to 4 persons	311	64.0 (59.2-68.6)
	≥5 persons	173	36.0 (31.4-40.8)
Children in the household	No	169	33.9 (29.3-38.8)
	Yes	315	66.1 (61.2-70.7)
Knowledge of dengue	Low	19	4.5 (2.7-7.5)
	High	465	95.5 (92.5-97.3)
Experience of dengue	No experience	211	41.5 (33.8-49.5)
	Someone known	148	32.7 (27.4-38.5)
	Self/household member	125	25.8 (20.8-31.6)
Aware of *Wolbachia* program	No	369	76.6 (69.9-82.2)
	Yes	115	23.4 (17.8-30.1)
Knowledge of *Wolbachia*	Low	422	88.2 (81.6-92.7)
	High	62	11.8 (7.3-18.4)

CI = confidence interval

Knowledge of dengue and source of information

Almost all respondents (98.7%) reported having obtained information regarding dengue and 95.5% obtained a high score on the subject. Around half (52.7%) obtained the information from health care providers, followed by television (48.7%) and community meeting (46.6%). Among all respondents, 39.5% reported having intentionally sought information regarding dengue with health-care providers as the predominant source (47.2%). A quarter (25.8%) claimed to have first-hand experience of dengue infection, either themselves or of a household member, while 41.5% reported that they did not know anyone who had experienced dengue infection.

Almost all respondents could recognize fever as a symptom of dengue (97.1%) but fewer recognized nausea (25.8%), bleeding (17%), and rash (18.1%) as symptoms of the disease. The majority recognized that dengue was transmitted by mosquito bites (93.6%); however, only a few knew that it was caused by a virus (2.9%). Although the majority knew that dengue could be deadly (97.5%), most believed that early treatment could prevent its severity (95.6%).

Planned action upon perceiving dengue infection

Most respondents reported that they would visit a healthcare provider (84.4%) and 70.7% of the respondents would seek medical help within 48 hours after the onset of perceived dengue symptoms. A half would first visit a public health center (51.6%) according to the established administrative procedure (38.2%) or because of the short distance from home (27.4%). The most reported symptom that would lead to a health facility visit was fever (97.0%), followed by nausea/vomiting (25.8%) and rash (18.1%).

Awareness and knowledge of the Wolbachia-infected Ae. aegypti Program

Among respondents, only a quarter (23.4%) were aware of the current *Wolbachia*-infected *Ae. aegypti* release in their area before the interview and only 11.8% obtained a high score on the subject (Table 1). A minority knew that *Wolbachia* was a bacterium (4.5%) that could be passed on to the *Ae. aegypti* offspring (15.4%) and could not be transmitted to humans (11.5%).

Awareness was higher among those with ≥5 household members (29.9% vs 19.7%), those without an open space around the house (26.5% vs 15.3%), and those with higher income (27.0% vs 15.7%) (Table 2). Around two fifths (40.9%) of those who were aware of the *Wolbachia* program obtained the information from mass meetings and around a quarter obtained the information from local health volunteers (26.8%), community leaders (28.8%), or the World Mosquito Program (WMP) staff (25.3%).

Table 2 Initial awareness of *Wolbachia*-infected *Ae. aegypti* program. (n=484)

Variable	Category	Weighted % awareness (95% CI)	P-value*
Overall (n=115)		23.4 (17.8-30.1)	
Sex	Male	24.1 (17.3-32.7)	0.688
	Female	22.7 (16.9-29.9)	
Age	21-40 years	21.2 (12.9-32.4)	0.370
	41-60 years	25.6 (19.6-32.8)	
	>60 years	19.9 (12.4-30.4)	
Marital status	Single/widowed	25.8 (15.7-39.2)	0.667
	Married	23.9 (17.1-30.3)	
Household members	1 to 4 persons	19.7 (14.6-26.2)	0.011
	≥5 persons	29.9 (21.5-39.7)	
Open space around house	No	26.5 (19.5-34.9)	0.041
	Yes	15.3 (9.5-23.7)	
Knowledge of dengue	Low	10.2 (1.4-47.2)	0.270
	High	24.0 (18.3-30.9)	
Previous experience of dengue	No experience	26.8 (18.1-37.7)	0.233
	Someone known	18.1 (11.8-26.9)	
	Self/household member	24.5 (17.1-33.7)	
Income/month	<1,846,400	15.7 (10.8-22.2)	0.017
	≥1,846,400	27.0 (19.7-35.9)	

*CI = confidence interval. * P-value from design-effect adjusted Rao-Scott F test.*

Perceived harmful effects of the Wolbachia-infected Ae. aegypti

Among all respondents, a majority (81.4%) doubted whether Wolbachia could reduce dengue transmission, and 1.4% believed that transmission could not be reduced by the release of mosquitoes. Only 17.2% believed that the program could reduce dengue transmission. Concerning the safety issue, a majority stated they were unsure about the possible harmful effects on human health (63.8%) or were definitely concerned about the risk to human health (17.7%); 17.5% believed there was no risk to their health. Overall, 46.8% perceived a recent change in mosquito population. Among them a majority reported an increase in mosquito population in the evening (56.4%), followed by an increase in mosquito population in the daytime (28.5%) (Table 3).

Table 3 Perception of *Wolbachia*-infected *Ae. aegypti* program and perceived recent change in mosquito population (n=484)

Variable	Number	Weighted % (95% CI)
Wolbachia-infected *Ae. aegypti* reduces dengue transmission		
No	6	1.4 (0.6-3.2)
Unsure	390	81.4 (74.3-86.8)
Yes	88	17.2 (12.0-24.0)
Wolbachia-infected *Ae. aegypti* affects humans' health		
No	86	17.5 (12.5-24.0)
Unsure	310	64.8 (58.4-70.6)
Yes	88	17.7 (13.7-22.6)
Perceived recent change in mosquito population		
No	83	17.4 (11.9-24.7)
Unsure	189	35.8 (28.6-43.6)
Yes	212	46.8 (38.6-55.2)
Direction and time of change in mosquito population * (n=212)		
Increase in daytime	54	28.5 (18.6-41.1)
Increase in evening	118	56.4 (46.9-65.4)
Decrease in daytime	44	18.5 (12.1-27.3)
Decrease in evening	34	15.6 (10.1-23.3)

* *Times of perceived change not mutually exclusive. CI = confidence interval*

Change in mosquito population was more commonly reported by those who were initially aware of the program (63.9%) than by those who were unaware (41.6%). Among the 115 respondents who were already aware of the *Wolbachia* program, 42.1% had concern on the possible harmful effects of the implementation of the program, and among those who were initially unaware, 63.1% expressed some concern over possible harmful effects (p=0.004) (Table 4). Concern over possible harmful effects was also higher among respondents whose house was adjacent to an open vegetated space (70.2% vs 53.5%, p=0.015) and those with a lower knowledge about *Wolbachia* (62.0% vs 29.2 %, p<0.001).

Table 4 Concern over possible harmful effects of *Wolbachia*-infected *Ae. aegypti* program (n=484)

Variable	Category	Weighted % concern (95% CI)	p-value*
Overall (n=274)		58.2 (51.3-65.0)	
Initially aware of the *Wolbachia* program	No	63.1 (55.5-70.1)	0.004
	Yes	42.1 (30.5-54.7)	
Age	21-40 years	55.3 (43.7-66.4)	0.032
	41-60 years	55.0 (47.2-62.5)	
	>60 years	68.6 (57.6-77.9)	

Variable	Category	Weighted % concern (95% CI)	p-value*
Household members	1 to 4 persons	62.9 (55.9-70.2)	0.017
	≥5 persons	49.8 (40.0-59.5)	
Open space around house	No	53.5 (45.5-61.2)	0.015
	Yes	70.2 (58.5-79.7)	
Knowledge of dengue	Low	74.7 (51.7-89.0)	0.094
	High	57.4 (50.2-64.3)	
Previous experience of dengue	No experience	54.9 (45.2-64.2)	0.301
	Someone known	63.7 (52.1-73.9)	
	Self/household member	56.5 (47.2-65.3)	
Knowledge of *Wolbachia*	Low	62.0 (54.9-68.6)	<0.001
	High	29.2 (16.7-45.7)	
Perceived recent change in mosquito population	No change	57.6 (43.3-71.9)	0.250
	Decreased	48.4 (37.8-59.1)	
	Increased	66.3 (50.7-79.0)	
	Unsure	54.5 (44.0-64.5)	
Wolbachia perceived to reduce dengue transmission	Yes	38.2 (25.2-53.2)	0.005
	Unsure/no	62.3 (54.8-69.2)	
Wolbachia perceived to affect health	No	40.6 (27.6-55.0)	0.005
	Unsure/yes	61.9 (54.7-68.6)	

*CI = confidence interval. * P-value from design-effect adjusted Rao-Scott F test.*

Change in dengue prevention routine after Wolbachia-infected Ae. aegypti release

Among all respondents, the majority had not changed their routine in performing dengue prevention activities (76.8%). Of the 115 respondents who were initially aware of the *Wolbachia*-infected *Ae. aegypti* release, only 20 reported changing their dengue prevention activities after the program had been implemented (17.2%). Seven started cleaning the house and surroundings, four started removing unused items that were potential mosquito breeding sites, and four started cleaning water containers. Meanwhile, both mosquito repellents and fishes were no longer used by four respondents.

Respondents were asked to give comments regarding the release of *Wolbachia*-infected *Ae. aegypti* and the current survey using an open question. One third stated that they supported the release of *Wolbachia*-infected *Ae. aegypti* (35.2%) and wanted to receive more information regarding the program (38.1%). A few (6.8%) requested that additional mosquito control programs be conducted in their neighborhood, e.g., the placement of additional MRCs (3.5%), performing fogging (2.7%), and giving larvicides (0.6%). Of the respondents, 14.3% raised concern regarding the danger of dengue and a few were concerned about mosquito nuisance (5.6%) from the placement of the mosquito release containers.

Factors associated with awareness of Wolbachia-infected Aedes aegypti

Logistic regression models were constructed based on the DAG shown in Figure 1 to identify factors associated with awareness of the *Wolbachia*-infected *Ae. aegypti* control program.

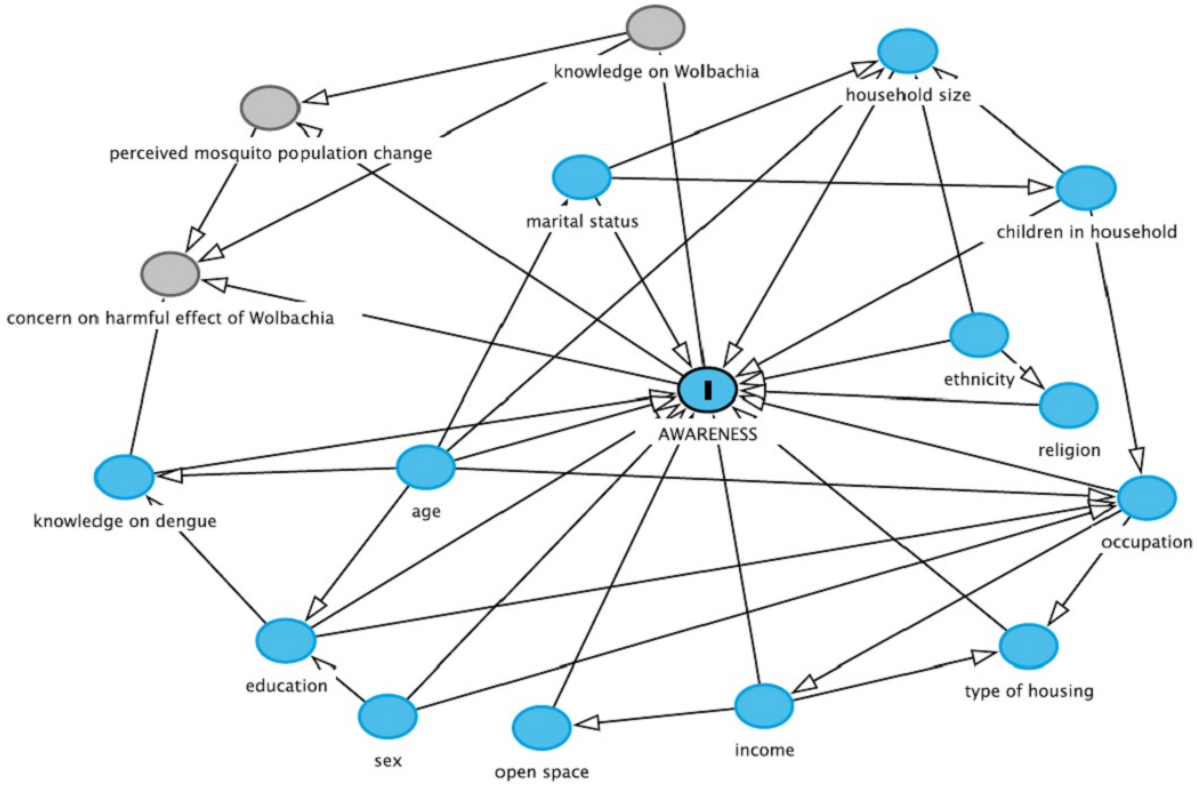

Figure 1 Directed acyclic graph showing potential causal pathways between exposures and awareness of the *Wolbachia*-based control program

Table 5 shows that awareness was inversely associated with having a smaller household size (aOR=0.50, 95% CI=0.35-0.88), lower income (aOR=0.47, 95% CI=0.26-0.85), and having an open space with vegetation around the house (aOR=0.51, 95% CI=0.27-0.98).

Table 5 Factors associated with awareness of *Wolbachia*-infected *Ae. aegypti* program. (n=484)

Variable	Being aware of *Wolbachia*-infected *Ae. aegypti*		
	Adjusted OR (95% CI)	p-value*	Adjustment set
Sex		0.688	-
Male	1		
Female	0.92 (0.63-1.36)		
Age		0.381	-
21-40 years	1		
41-60 years	1.29 (0.74-2.25)		
>60 years	0.93 (0.50-1.73)		
Household members		0.016	age, eth, mst, chd
≥5 persons	1		
1-4 persons	0.50 (0.35-0.88)		
Income (USD/month)		0.017	ocp

Variable	Being aware of *Wolbachia*-infected *Ae. aegypti*		
	Adjusted OR (95% CI)	p-value*	Adjustment set
≥120	1		
<120	0.47 (0.26-0.85)		
Open space around house		0.044	inc
No	1		
Yes	0.51 (0.27-0.98)		
Knowledge of dengue		0.434	age, edu
High	1		
Low	0.48 (0.07-3.45)		

Abbreviations:
age = age; ocp = occupation; mst = marital status
eth = ethnicity; inc = income; chd = children in household
edu = education level

OR = odds ratio. CI = confidence interval.
*Values in brackets represent 95% confidence interval. *Wald test.*

Factors associated with concern over possible harmful effects of Wolbachia-infected Ae. aegypti

Logistic regression models were constructed to identify factors associated with concern over possible harmful effects of *Wolbachia*-infected *Ae. aegypti* control program (Figure 2).

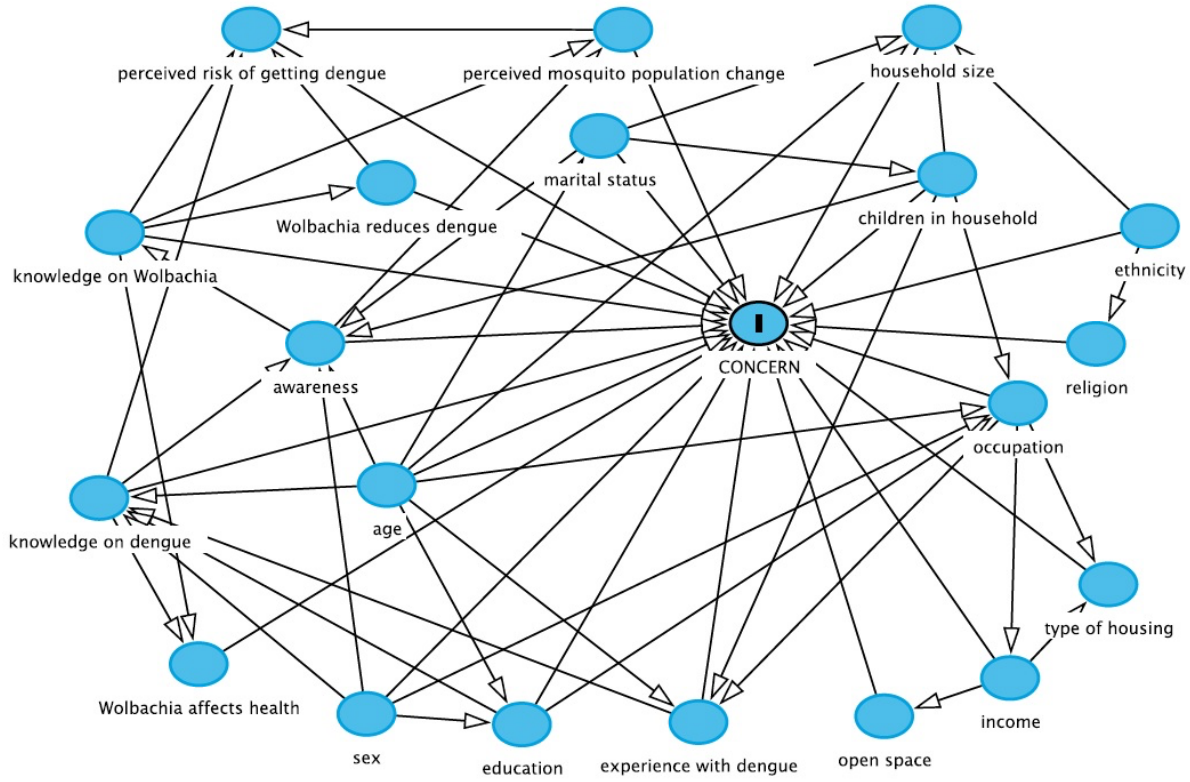

Figure 2 Directed acyclic graph showing potential causal pathways between exposures and concern over possible harmful effects of the *Wolbachia*-based program

As shown in Table 6, concern over possible harmful effects was positively associated with older age, (aOR=1.76, 95% CI=1.04-2.99) for ≥60 vs 21-40 years), smaller household size (aOR=1.86, 95% CI=1.17-2.95), lower monthly income (aOR=1.63, 95% CI=1.04-2.57), having an open space with vegetation around the house (aOR=2.02, 95% CI=1.16-3.51)), not being aware of the *Wolbachia* program (aOR=2.26, 95% CI=1.28-3.98), low knowledge about *Wolbachia* (aOR=3.00, 95% CI=1.38-6.53), and perceived recent change in mosquito population (aOR=2.94, 95% CI=1.43-6.03 for increased vs decreased

Table 6 Factors associated with concern over possible harmful effects of *Wolbachia*-infected *Ae. aegypti* program. (n=484)

Variable	Concern over possible harmful effects		
	Adjusted OR (95% CI)	p-value*	Adjustment set
Sex		0.162	-
Male	1		
Female	1.26 (0.92-1.73)		
Age		0.038	-
21-40 years[a]	1		
41-60 years	0.99 (0.64-1.53)[a]		
>60 years	1.76 (1.04-2.99)[b]		
Household members		0.013	age, eth, mst, chd
≥5 persons	1		
1-4 persons	1.86 (1.17-2.95)		
Income (USD/month)		0.041	occ
≥120	1		
<120	1.63 (1.04-2.57)		
Open space around house		0.017	inc
No	1		
Yes	2.02 (1.16-3.51)		
Knowledge of dengue		0.334	sex, age, edu, dxp
High	1		
Low	1.57 (0.62-3.98)		
Aware of *Wolbachia*-program		0.008	sex, age, mst, chd, dkl,
Yes	1		
No	2.26 (1.28-3.98)		
Knowledge of *Wolbachia*		0.008	win
High	1		
Low	3.00 (1.38-6.53)		
Perceived recent change in mosquito population		0.029	win, wkl
Decreased[a]	1		
No change	1.55 (0.74-3.25)[a]		

Variable	Concern over possible harmful effects		
	Adjusted OR (95% CI)	p-value*	Adjustment set
Increased	2.94 (1.43-6.03)[b]		
Unsure	1.15 (0.62-2.14)[a]		
Wolbachia perceived to reduce dengue transmission		0.422	wkl
Yes	1		
Unsure/no	1.34 (0.66-2.75)		
Wolbachia perceived to affect health		0.283	dkl, wkl
No	1		
Unsure/yes	1.38 (0.78, 2.44)		

Abbreviations:

sex = sex	occ = occupation	chd = children in household
age = age	inc = income	dkl = knowledge on dengue
eth = ethnicity	mst = marital status	win = aware of *Wolbachia* program
edu = education level	dxp = previous experience of dengue	wkl = knowledge on *Wolbachia*

OR = odds ratio. CI = confidence interval.
*Values in brackets represent 95% confidence interval. *Wald test.*
[ab] Values within variables and outcome not having a superscript in common differ significantly

DISCUSSION

Similar to the findings of previous studies,[41-43] most respondents were reasonably well-informed about the vector of dengue disease, but few were aware that the disease was due to a virus. The majority knew that a person with dengue might manifest fever, but less than a quarter recognized that nausea/vomiting, bleeding, and rash might be the symptoms of dengue. In parallel with previous studies,[44,45] most of the respondents stated they would visit a healthcare facility when perceiving dengue, mostly within a two-day period from the onset of symptoms. The gaps in knowledge of dengue symptoms indicated that in addition to covering the cause and vector of the disease, educational programs on dengue should aim to improve knowledge of dengue symptoms.

Our study found that only around one quarter of respondents were aware of the implementation of the *Wolbachia*-based program in their area, even though an extensive community engagement process was performed to inform the general public and community stakeholders prior to the placement of the *Wolbachia*-infected *Ae. aegypti* mosquito release containers, which had been done two years before this study.[34] Previous studies similarly found that long-term health education programs were insufficient in improving awareness and health behavior.[46,47] However, in this study, the awareness was assessed using only a single unprompted question. Respondents' awareness was not evaluated after being given an explanation regarding the *Wolbachia*-based dengue control, and their lack of awareness may have been due to a lapse of memory. For this reason, our survey might have captured a lower awareness of the respondents, as a previous survey from Australia found that the awareness was higher after giving

prompted information regarding the program.[25]

More than half of the respondents raised some concern over the safety and effectiveness of *Wolbachia*-infected mosquito release program. A previous study in Vietnam similarly showed that the community was concerned about the safety of this method, i.e., the safety of getting bitten by a *Wolbachia*-infected mosquito and the possibility of *Wolbachia* being transmitted to a human, and expressed worries that *Wolbachia*-infected mosquitoes might cause or transmit other diseases.[26] By contrast, the present study found that only a few respondents (5.6%) expressed concern about an additional mosquito nuisance, which is much lower than that in a study of community perception of releasing genetically modified mosquitoes in Florida, where 51% felt that the mosquitoes were a nuisance.[33] Differences in the mosquito release strategy in Yogyakarta compared to that in Florida and Vietnam might influence this low perceived nuisance as, in Yogyakarta, the release was performed by placing eggs inside the containers in outdoor areas and hatched within a two-week period, while in Florida and Vietnam, this process was implemented by releasing adult mosquitoes.[26,33-35] The community concerns expressed by the respondents indicated that it is important to consider other approaches that may be more effective for maintaining information regarding the safety and effectiveness of the *Wolbachia*-infected *Ae. aegypti* release.

Despite an added mosquito nuisance not figuring prominently among our respondents, there was an association between perception of a recent increase in numbers of mosquitoes and concern over the possible harmful effects of *Wolbachia*-infected mosquitoes, even among those respondents not initially aware of the existence of the program. In contrast, in a study in another area of Yogyakarta, Sleman and Bantul districts, where *Wolbachia*-infected mosquitoes were previously released, it was found that residents reported no noticeable increase in mosquito numbers.[24] This finding should help to shape the communication strategy, particularly for the new communities where the implementation of *Wolbachia*-based mosquito control is planned, to help manage the concern over possible harmful effects.

The release of *Wolbachia*-infected mosquitoes was introduced as an additional method for preventing dengue, and the residents of Yogyakarta were encouraged to continue performing their existing dengue prevention activities.[34] Consistent with this intention, our study found that although *Wolbachia*-infected *Ae. aegypti* had been released, only a few respondents who were aware of the control program reported changing their dengue prevention routine, mostly by upgrading the cleaning of house and surroundings.

The extent to which the awareness and concern over possible harmful effects revealed in our study reflect those of the whole community, should be considered in view of the limitation due to the availability of household members during the visits. Although our respondents appeared to represent the urban population in terms of religion, education level and marital status, they may not have well represented the composition of the community residents and is likely to have over-represented those residents who stayed or worked at home during the day, who are likely to be older. Indeed, a large proportion was aged over 40. Nevertheless, the low proportion of awareness of the control program showed only a slight drop across the increasing age groups. As information regarding the

control program was obtained mainly from community meetings, WMP staff and local health volunteers, those residents staying at home may have had less contact with these sources of information. The relatively long interval between first implementation of the program and the time of the interviews may have been a cause of forgetfulness regarding information already received.

In conclusion, the percentage of respondents who were aware of the local implementation of the *Wolbachia*-infected mosquito release was low after two years of its implementation. The high proportion of residents that expressed concern about the possible harmful effects of the *Wolbachia*-infected mosquito release, especially among those with low awareness and/or low knowledge of the *Wolbachia*-based mosquito control program, implies that vigorous efforts should be taken to improve the retention of information about *Wolbachia*-infected *Aedes aegypti* the activities of the control program, and the safety of humans from the infected mosquitoes.

RECOMMENDATION

There is a need to improve educational programs focusing on the cause and symptoms of dengue as there are gaps in knowledge on this information. Assessment of awareness of the dengue control program should be performed soon after the program being implemented, so that the stakeholders can evaluate the effectiveness of the program dissemination. The findings of previously released areas are also important to shape the communication strategy for future implementation.

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