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Research Article
Presence of Arboviral Vector in Tourist Attractions and Public Places: Abundance, Associated Factors and Susceptibility to Temephos

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Abstract
Background and Objective: *Aedes* mosquito is a competent vector of arboviruses, mainly dengue, chikungunya, Zika and yellow fever. The high incidence of arboviral infections among travelers indicated the vulnerability of tourist attractions and public places visitors to arboviral infections. This study aimed to determine the presence and population density of *Aedes* mosquitoes at the places, as well as the susceptibility to temephos larvicide. **Materials and Methods:** A total of 17 tourist attractions and public places in four districts/cities of Central Java Province were selected as study sites and 25-40 ovitraps were installed in each location for three days. The coordinates, altitude, vegetation density, shade, wind speed, air temperature and humidity were recorded. Ovistrips and water of each ovitrap were observed in the laboratory to determine the ovitrap index (OI) and egg density index (EDI), mosquito species and their susceptibility to temephos. **Results:** A total of 12,231 eggs were obtained with a range, OI and EDI of 0-1,024, 42% and 31.93 eggs/trap, respectively. Vegetation density, shade and wind speed were significantly associated with the presence of *Aedes* eggs in the ovitraps ($p<0.05$), while altitude, temperature and air humidity were correlated with the average of *Aedes* eggs per ovitrap. **Conclusion:** Although *Aedes* larvae in all study sites were susceptible to temephos 0.02 mg/L the results indicated the vulnerability of visitors to *Aedes*-borne diseases. Further investigation of arbovirus infections in *Aedes* mosquitoes at tourist attractions and public places is necessary conducted to evaluate the potential and risk of pathogen transmission to visitors.

Key words: *Aedes* mosquito, tourist attractions, public places, temephos, arboviral infections

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

INTRODUCTION

Aedes mosquitoes, namely *Ae. aegypti* and *Ae. albopictus* are competent primary and secondary vectors in arboviral infections such as dengue, chikungunya, Zika and yellow fever[1,2]. The number of these *Aedes*-borne infections has escalated progressively every year and become a global public health problem[3]. The incidence rate of arboviral diseases has reached 591/100,000 populations[4] and caused 700,000 deaths per year[5] in which the sociodemographic, environmental conditions, viral genetics and abundance of competent vector populations have been identified as influential factors[1,6]. The risk of transmission of *Aedes*-borne diseases is increasing due to extreme climate change and forming two different poles where Europe is more exposed to *Ae. aegypti*-borne viruses, while Southeast Asia and the West Pacific are exposed to *Ae. albopictus*-borne diseases[7,8].

Dengue, Zika and chikungunya are also prevalent among travelers[9,10] with varying incidence rates in different countries. Dengue infections involved 4.6% of travelers visiting parks and green spaces in Japan[11], 71% of European travelers returning from Asia[12] and even a spike of up to 168% occurred among travelers in the United States of America in 2019 from the previous annual average of cases[13]. Co-infection of these three arboviruses was also detected from Dutch tourists visiting Suriname with incidence rates (IR) of 47.0, 11.6 and 5.6 per 1,000 person-months for dengue, Zika and chikungunya, respectively[14]. This situation indicates that tourist attractions, parks and green spaces have a high potential for arbovirus transmission[15]. Several studies have proven that green spaces, parks and public spaces in urban areas are preferred habitats for *Aedes* mosquito populations[16], in which *Ae. albopictus* species is more dominant than *Ae. aegypti*[17]. The existence and abundance of the *Aedes* mosquito population in an area is a risk factor for arboviral infections[18]. In dengue transmission, the virus could be detected in mosquito vectors up to 14 days after a new case is detected in an area[19]. The population density of *Aedes* mosquitoes varies according to environmental conditions whereby the presence of breeding sites, air temperature and humidity and green areas have an influence[17,20,21]. The density of *Aedes* eggs was found to be higher in areas with denser vegetation[22]. Hence, the presence of vegetation around residences increases the risk of mosquito exposure and arboviral infection[23], although wind speed prevents the activity and spread of *Aedes* mosquitoes[24].

Several studies reported on the existence of *Aedes* mosquitoes in tourism destinations. A study in the Bang Kachao Riverbend ecotourism area, in Thailand has demonstrated on higher density of immature *Aedes* in indoor breeding habitats as compared to outdoor[25]. In contrast, the prevalence of breeding habitat and positive larval habitat of *Aedes* was higher in parks than in residential areas in Ho Chi Minh City, Vietnam whereby the vegetation shade was found to be correlated with *Aedes* mosquito density, especially *Ae. albopictus*[26]. In Indonesia, high *Aedes* indices have been recorded in the Pangandaran Tourism Market[27] and public places in Semarang[28]. Data for 2022 stated that Indonesia has 2,930 commercial tourist attractions, most of which are nature tourism, parks and open spaces[29], but information about the presence of dengue vectors and arboviruses in these places was limited. Therefore, this study aimed to determine the emergence, population density and insecticide resistance of *Aedes* mosquitoes in tourist attractions and public places which is useful for evaluating the vulnerability and need for efforts to prevent and control arbovirus transmission.

MATERIALS AND METHODS

Study sites: Five types of tourist attractions were chosen in this study namely beach/coastal areas, gardens, natural scenes, cultural sites and zoos in Central Java Province, Indonesia. Seventeen locations in three districts (Semarang, Kendal and Jepara) and one city (Semarang) representing seven types of tourist destinations were selected, namely Bandengan, Bondo, Blebak, Marina, Ngebum, Sendang Sikucing and Tirang (beaches); Kendal City and Unimus (garden), Palagan Ambarawa Monument and Portuguese Fort (historical building), Central Java Grand Mosque and Sam Poo Kong Temple (worship place), Gedong Songo Temple (cultural building), Songgo Langit Waterfall (natural scene) and Semarang Zoo were selected. These study sites also represented the coastal, inland and hilly areas. The research was carried out after a survey permit was obtained from the tourism office in each district/city.

Ovitrap installation and mosquito detection: The emergence of mosquitoes in those sites was detected using an oviposition trap (ovitrap). The device was made of a black plastic cup, filter paper ovistrip and tap water. The ovitraps were placed in several parts of each study site near the water containers for three days. A total of 25 ovitraps were installed in each location except at Semarang Zoo and Unimus Garden, with 40 ovitraps each. All ovitraps were retrieved on the third day and brought to the laboratory. The ovistrip was removed from each existing ovitrap and dried in a shady place. The water in the ovitrap was poured into a plastic tray and placed in the entomology laboratory to determine the appearance of any mosquito larvae. The dried ovistrips were examined under the microscope to determine and count the presence of *Aedes* mosquito eggs.

Emergence and population density of *Aedes* mosquito: *Aedes* mosquito emergence in each study site was determined by the existence of mosquito eggs in the ovistrip and mosquito larvae in the water of the ovitrap, while the population density was measured based on the ovitrap index (OI). This index described the proportion of eggs or larvae in positive ovitraps over the existing ones.

Environmental conditions: The coordinate and elevation data of each study site were recorded using the Geographic Positioning System (GPS) handheld device, Garmin 72H to determine the geographical and altitudinal distribution of the study sites. Several environmental factors like air temperature and humidity, altitude, vegetation density, shadiness and wind gusts were also observed. Air temperature and humidity were recorded using HAAR-SYNTH hygrometer, while vegetation density was subjective-visually determined according to the criteria for the number of trees in a 100 m square (trees/hectare) below $\leq$20 (very low), 21-50 (low), 51-100 (medium), 101-200 (high) and >200 (very high)[30]. The shadiness condition was classified into high, medium, low and very low based on the width of shaded areas in the study sites.

Detection of insecticide resistance: The susceptibility of *Aedes* mosquito larvae to temephos larvicide was monitored by applying the WHO procedures using a diagnostic concentration of 0.02 ppm. As many as twenty-third or early fourth-instar larvae were exposed to 0.02 mg/L temephos solution in 100 ml of distilled water for 24 hrs. Mortality was calculated from the percentage of larvae that died with the provisions being susceptible if >98%, tolerant if 90-98% and resistant if <90%. This experiment used three replicates and one control group (without temephos solution). If there was 5-20% larval death in the control group, then the experiment was corrected using Abbott's formula, whereas if >10% pupae were formed or >20% death was recorded, then the experiment must be repeated[31].

Data analysis: Data analysis was carried out descriptively and analytically using SPSS software 17.0 version. The results of data analysis are displayed in the form of tables, graphs and maps according to the research objectives.

Ethical approval: This study obtained the Ethical Approval Certificate from the Health Research Ethics Commission of Public Health Faculty of Universitas Muhammadiyah Semarang number: 071/KEPK-FKM/UNIMUS/2023.

RESULTS

Out of 455 ovitraps placed in all study sites, 383 of them (84.2%) have been retrieved (Table 1). All ovitraps placed at five locations, namely Sam Poo Kong Temple, Bandengan Beach, Bondo Beach, Portuguese Fortress and Songgo Langit Waterfall were completely retrieved. A range of 1-17 ovitraps (4-68%) were lost in 12 locations with the highest loss being at Marina Beach while the lowest was at Kartini Beach, Sendang Sikucing Beach and Kendal City Garden. This data shows that ovitraps are suitable to be used for the survey of mosquito vectors at the study sites selected.

The presence and activity of *Aedes* mosquitoes were detected in 13 tourist attractions and public places (76.5%) with the range and average ovitrap index of 0-100% and 42.0% (Table 2). The five locations with the highest ovitrap index were Semarang Zoo, Kendal City Garden, Unimus Garden, Portuguese Fortress and Blebak Beach. There were four tourist destinations where mosquito eggs were not found and all of them were beaches. These findings indicate *Aedes* mosquitoes' presence in most tourist attractions and public places selected. The emergence and egg-laying activity of *Aedes* mosquitoes in these places is an indicator of the vulnerability of visitors to these tourist attractions to arbovirus transmission.

A total of 12,231 *Aedes* mosquito eggs were found in 161 (42%) of 383 retrieved ovitraps with a range and mean of 0-1024 and 31.93 eggs from all existing ovitraps (Table 3). The highest and lowest average number of *Aedes* eggs in the ovitrap were found at Semarang Zoo and Kartini Beach, while the highest and lowest average number of larvae in the water in the ovitrap were found at Semarang Zoo and Palagan Ambarawa Monument, respectively. Semarang Zoo is the only location where *Aedes* eggs were detected in all existing ovitraps. On the other hand, only *Aedes* larvae were found in the ovitrap retrieved from Gedong Songo Temple. The co-occurrence of *Aedes* mosquito eggs and larvae was demonstrated in ovitraps placed in 52.9% of tourist destinations and public places. Generally, *Aedes* eggs were found in all five types of tourist attractions selected in this study (Fig. 1).

This study recorded variations in regional altitude, air temperature and air humidity at the study sites with a range and mean of 2-1,316 and 113.32 masl, 22-36 and 28.93°C and 10-88 and 64.62%, respectively. The lowest altitude is observed at Tirang Beach, while the highest altitude is at Gedong Songo Temple. The lowest air temperature was recorded at Gedong Songo Temple and Sam Poo Kong Temple, while the highest was recorded at

Table 1: Distribution of installed, retrieved and lost ovitraps placed at selected tourist destinations

		Ovitrap retrieval			
		Retrieved		Lost	
Tourist destination	Installed ovitraps	n	%	n	%
Sam Poo Kong Temple	25	25	100.0	0	0.0
Central Java Grand Mosque	25	16	64.0	9	36.0
Tirang Beach	25	13	52.0	12	48.0
Marina Beach	25	8	32.0	17	68.0
Semarang Zoo	40	33	82.5	7	17.5
Unimus Garden	40	35	87.5	5	12.5
Gedong Songo Temple	25	16	64.0	9	36.0
Palagan Ambarawa Monument	25	23	92.0	2	8.0
Ngebum Beach	25	20	80.0	5	20.0
Sendang Sikucing Beach	25	24	96.0	1	4.0
Kendal City Garden	25	24	96.0	1	4.0
Kartini Beach	25	24	96.0	1	4.0
Bandengan Beach	25	25	100.0	0	0.0
Blebak Beach	25	22	88.0	3	12.0
Portuguese Fort	25	25	100.0	0	0.0
Bondo Beach	25	25	100.0	0	0.0
Songgo Langit Waterfall	25	25	100.0	0	0.0
Total	455	383	84.2	72	15.8

Table 2: Emergence of mosquito eggs in existing ovitraps

		Mosquito eggs emergence			
		Yes		No	
Tourist destination	Existing ovitraps	n	%	n	%
Sam Poo Kong Temple	25	11	44.0	14	56.0
Grand Mosque of Central Java	16	6	37.5	10	62.5
Tirang Beach	13	2	15.4	11	84.6
Marina Beach	8	0	0.0	8	100.0
Semarang Zoo	33	33	100.0	0	0.0
Unimus Garden	35	27	77.1	8	22.9
Gedong Songo Temple	16	2	12.5	14	87.5
Palagan Ambarawa Monument	23	5	21.7	18	78.3
Ngebum Beach	20	7	35.0	13	65.0
Sendang Sikucing Beach	24	0	0.0	24	100.0
Kendal City Garden	24	22	91.7	2	8.3
Kartini Beach	24	4	16.7	20	83.3
Bandengan Beach	25	0	0.0	25	100.0
Blebak Beach	22	15	68.2	7	31.8
Portuguese Fort	25	19	76.0	6	24.0
Bondo Beach	25	0	0.0	25	100.0
Songgo Langit Waterfall	25	10	40.0	15	60.0
Total	383	161	42.0	223	58.0

Marina and Tirang Beach. The lowest air humidity was recorded at Marina and Tirang Beaches, while the highest was recorded at Unimus Garden. These three physical factors correlated significantly with the number of *Aedes* eggs in the ovitrap, in which the higher the altitude (Fig. 2a), the fewer *Aedes* eggs were found in the ovitraps ($r = -0.106$, $p = 0.000$). Conversely, the higher the temperature ($r = 0.155$, $p = 0.000$) and air humidity ($r = 0.215$, $p = 0.000$), the more *Aedes* eggs were found in the ovitraps Fig. 2(b-c).

Vegetation density, shade and wind gusts also contributed significantly to the percentage of *Aedes* eggs detected in the ovitraps. High vegetation density and low-category wind gusts were observed in the parks, natural sites and zoos where high densities of *Aedes* eggs were found. High temperatures and fast wind gusts were recorded at the beaches. The highest shade was demonstrated at natural sites and zoos, while the lowest was observed at the beaches (Table 4). Bivariate analysis showed that vegetation density, shade and wind gust speed were significantly associated with the presence of *Aedes* eggs in the ovitraps ($p<0.05$), in which plant density and shade were positively associated, while wind gusts were negatively associated (Table 5). These results showed that the existence of more plants and shady places with lower wind gusts increased the number of positive ovitraps with *Aedes* eggs.

Apart from revealing the presence and distribution of *Aedes* mosquitoes in tourist destinations and public places, this study also detected the susceptibility of *Aedes* mosquito larvae to temephos larvicide. The bioassay test results showed that the mortality of *Aedes* larvae from all study sites reached 100% before 24 hrs of exposure (Table 3). This condition indicates that *Aedes* mosquito larvae in all study sites were fully susceptible to temephos larvicide.

Table 3: *Aedes* egg density in retrieved ovitraps based on tourist destinations and public places

		Aedes eggs density				Larval emergence*		
Tourist destination	Existing ovitraps	Min	Max	Mean	Std. Deviation	n	Average#	Larval mortality in bioassay Test to temephos (%)
Sam Poo Kong Temple	25	0	21	3.48	5.70	0	0.00	100
Grand Mosque of Central Java	16	0	49	6.13	13.53	0	0.00	100
Tirang Beach	13	0	7	0.92	2.29	5	0.63	100
Marina Beach	8	0	0	0.00	0.00	0	0.00	100
Semarang Zoo	33	27	593	210.91	167.97	363	27.92	100
Unimus Garden	35	0	1,024	97.97	186.49	66	2.75	100
Gedong Songo Temple	16	0	0	0.00	0.00	26	1.18	100
Palagan Ambarawa Monument	23	0	19	2.00	5.21	1	0.04	100
Ngebum Beach	20	0	13	1.70	3.44	0	0.00	100
Sendang Sikucing Beach	24	0	0	0.00	0.00	0	0.00	100
Kendal City Garden	24	0	85	37.88	29.14	336	13.44	100
Kartini Beach	24	0	10	0.75	2.21	2	0.08	100
Bandengan Beach	25	0	0	0.00	0.00	0	0.00	100
Blebak Beach	22	0	45	7.18	11.04	10	0.42	100
Portuguese Fort	25	0	54	16.40	17.43	8	0.00	100
Bondo Beach	25	0	0	0.00	0.00	0	0.00	100
Songgo Langit Waterfall	25	0	15	2.80	4.32	37	1.48	100
Total	383	0	1,024	31.93	96.76	854	2.23	100

*Larval emergence in the water of ovitrap and #Comparison between n and existing ovitraps

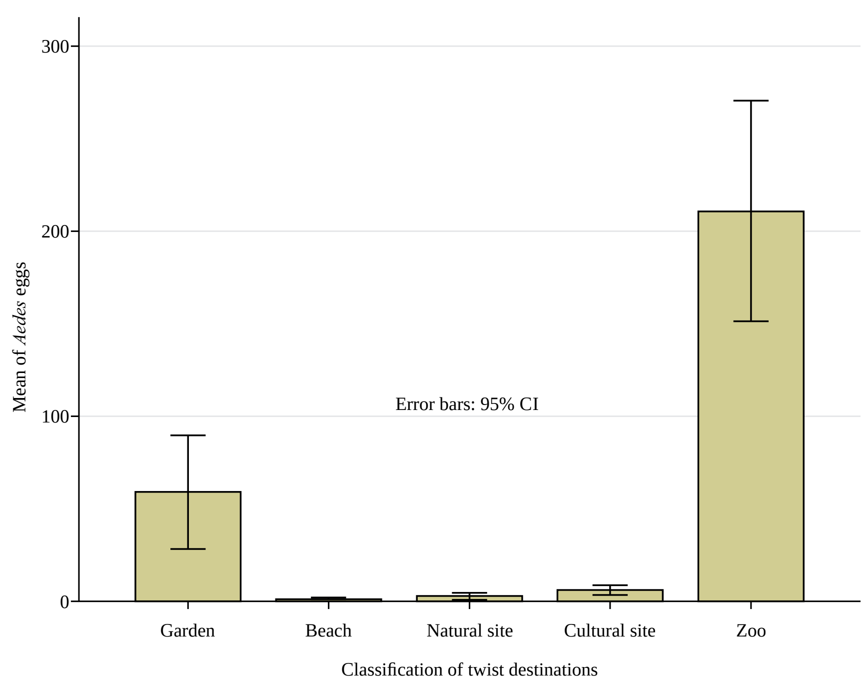

Fig. 1: Distribution of *Aedes* eggs based on the classification of tourist destinations

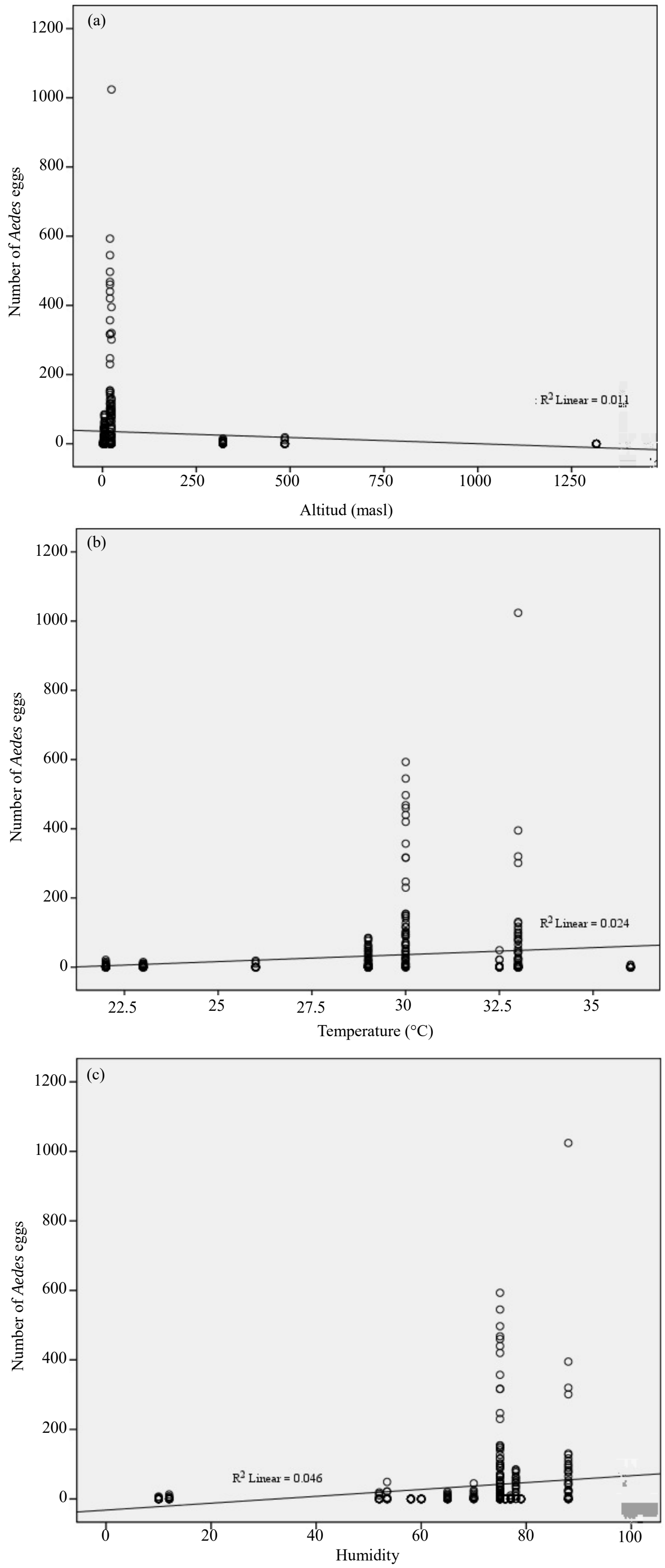

Fig. 2(a-c): Correlation between (a) Regional altitude, (b) Air temperature and (c) Air humidity with the number of *Aedes* eggs in positive ovitraps

Table 4: Distribution of *Aedes* eggs in positive ovitraps based on the environmental factors and study sites

	Study site classifications									
	Garden		Beach		Natural sites		Cultural sites		Zoo	
Environmental factors	n	%	n	%	n	%	n	%	n	%
Vegetation density										
High	59	78.7	22	13.7	25	100.0	0	0.0	33	100.0
Medium	0	0.0	25	15.5	0	0.0	64	71.9	0	0.0
Low	0	0.0	93	57.8	0	0.0	25	28.1	0	0.0
No/very low	16	21.3	21	13.0	0	0.0	0	0.0	0	0.0
Shadiness										
High	24	32.0	22	13.7	25	100.0	25	28.1	33	100.0
Medium	35	46.7	49	30.4	0	0.0	41	46.1	0	0.0
Low	16	21.3	24	24.9	0	0.0	23	25.8	0	0.0
No/very low	0	0.0	66	41.0	0	0.0	0	0.0	0	0.0
Wind breeze										
Fast	0	0.0	153	95.0	0	0.0	23	25.8	0	0.0
Medium	0	0.0	8	5.0	0	0.0	41	46.1	0	0.0
Slow	75	100.0	0	0.0	25	100.0	25	28.1	33	100.0

Table 5. Association between vegetation density, shade and wind gusts with *Aedes* eggs in positive ovitraps

	Aedes eggs in ovitraps				
	Positive		Negative		
Environmental variables	n	%	n	%	p-value
Vegetation density					
High	107	77.0	32	23.0	0.000
Medium	24	27.0	65	73.0	
Low	22	18.6	96	81.4	
No/very low	7	18.9	30	81.1	
Shadiness					
High	99	76.7	30	23.3	0.000
Medium	42	33.6	83	66.4	
Low	11	17.5	52	82.5	
No/very low	8	12.1	58	87.9	
Wind gusts					
Low	109	69.0	49	31.0	0.000
Medium	19	38.8	30	61.2	
High	32	18.2	144	81.8	

DISCUSSION

This study reveals the presence, density and geographical distribution of *Aedes* mosquitoes in tourist attractions and public places as an effort to expand the reach of arbovirus vector surveillance outside residential endemic areas. These findings produce important information to determine the presence and dynamics of mosquito vectors in specific target areas that have been found as the hotspots for important arboviral diseases. Typically, vector surveys and surveillance have been focusing on residential environments in areas where vector-borne diseases are endemic to monitor the presence and fluctuations of mosquito vectors, as a basis of control measures. This study is an effort to expand the surveillance of arbovirus vectors in tourist attractions and public places where information about the presence, density and distribution of *Aedes* mosquitoes in these places is still very limited. This effort is important to be carried out as some previous research reports have proven the occurrence of dengue, chikungunya and Zika transmission among tourists, especially after traveling from Latin America and Southeast Asia[13,32,33].

This study reveals that the majority of tourist attractions and public places in Central Java Province selected in this study were exposed to *Aedes* mosquitoes. The ovitrap index and the average number of eggs per ovitrap obtained in this study showed a lower density as compared to other study studies conducted in residential environments[34,35], in buildings[22] and in public places[25]. Apart from being relevant to other study reports and complementing information on the presence and condition of arbovirus vectors in Indonesia, these findings also confirmed that visitors of tourist attractions and public places are vulnerable to the transmission of arboviral diseases, especially dengue, chikungunya and Zika[33,36,37]. These findings supported several previous studies that indicated a risk factor for dengue transmission[6,18]. The abundance of *Aedes* eggs found in this study was higher than the results of other indoor surveys[22,38,39], but similar to the abundance of larvae in outdoor containers in Riau, Indonesia[34]. Air humidity and higher outdoor mosquito population densities are thought to be logical arguments for this finding[40]. The density of *Aedes* eggs in this study was lower than in other outdoor mosquito surveillance reports. Studies in Brazil found an egg density index of 1.5 higher[41], but reports of indoor studies in nearby areas found that *Aedes* egg densities were half times lower[22]. The result of this study was also similar to a study in Bandung City, West Java, Indonesia, in which the outdoor egg density index was six times higher than this finding[42].

The present study shows that the density of *Aedes* eggs in outdoor areas such as tourist attractions and public places varies according to specific local environmental conditions. Several environmental factors, especially vegetation density, shade, air humidity, wind speed and air temperature have a significant association with the ovitrap index and *Aedes* egg density. Shady, humid places, low wind gusts and air temperatures in the medium range are the preferred places for *Aedes* mosquitoes to oviposition. These findings proved that environmental factors play important roles in the survivorship of *Aedes* mosquitoes. Areas with denser vegetation[43] become shadier and the air humidity is higher[21]. The ovitrap index percentage and *Aedes* egg density were higher in these areas. This finding was similar to previous studies that highlighted the plant density, shaded areas, lighting and lower air temperatures that were preferred by mosquitoes in seeking places to lay their eggs[22]. This condition is under the physiological needs of *Aedes* mosquitoes in which the optimum temperature and humidity are 25-30°C and 60-90%, respectively. Inversely, they will stop their activities when temperatures are below 15°C and above 45°C and air humidity is below 40%[44]. High temperatures cause mosquitoes to become dehydrated and die, while low temperatures cause mosquitoes to have difficulty in sucking blood, disrupt their egg-hatching and the development of larvae into pupae[45]. High wind speeds (above 10 mph) inhibit mosquito flight activity thereby affecting host and oviposition sites[24], while low wind speeds are associated with vector activity which has an impact on increasing the incidence of dengue infection[46].

Aedes larvae from all study sites were shown to be susceptible to the organophosphate temephos larvicide. This finding was expected as these tourist attractions and public places are never been involved in any dengue vector control programs. Dengue vector control programs using temephos larvicide are more frequent in residential environments. Organophosphate temephos is still the larvicide of choice in controlling dengue vectors in endemic areas because of its effectiveness. Several studies have reported that *Aedes* larvae in residential environments are still susceptible to temephos both in Indonesia and in other countries[47-49].

CONCLUSION

Aedes eggs and larvae were found in 76.5% of tourist destinations and public spaces in Central Java Province with the OI and EDI of 42% and 31.93 eggs/trap, respectively. The presence of *Aedes* eggs in the ovitraps was associated with vegetation density, shade and wind speed, while the average number of eggs per ovitrap was determined by altitude, air temperature and air humidity. Five tourist destinations and public places with the highest OI were Semarang Zoo, Kendal City Garden, Unimus Garden, Portuguese Fort and Blebak Beach. The abundance of *Aedes* mosquitoes in tourist destinations and public spaces indicated the vulnerability of visitors of tourist attractions to the transmission of arboviral infections. However, *Aedes* larval populations at these tourist destinations and public places could still be effectively controlled by temephos larvicide based on their full susceptibility to this larvicide.

SIGNIFICANCE STATEMENT

This study reported the distribution and abundance of *Aedes* mosquitoes in tourist attractions and public places which can be important data in controlling arboviral transmission. This finding will help the researchers and public health workers uncover critical areas in providing alternative strategies for controlling mosquito vectors and further studies. These results reinforce that the arboviral vectors can be understood, monitored and controlled.

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