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Characteristics of water containers influencing the presence of *Aedes* immatures in an ecotourism area of Bang Kachao Riverbend, Thailand

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Abstract

Purpose – The purpose of this paper is to investigate the influence of breeding containers on the production of *Aedes* mosquitoes after a vector-control program in households that might support dengue transmission in tourist attraction areas of Bang Kachao Riverbend, Thailand.

Design/methodology/approach – A cross-sectional study was conducted in an ecotourism area of Bang Kachao Riverbend, Thailand, during the period October 2016 to September 2017. A total of 832 households from five villages participated in the study. Data collection employed an interview questionnaire, larval mosquito survey and measurements of the chemical properties of the water in each container. A binary logistic regression model was used to investigate the characteristics of water containers influencing the presence or absence of *Aedes* immatures.

Findings – The study found that water containers located inside households had a highly presence of *Aedes* immatures (2.22 times) compared with outdoor containers. Water containers without lids and water containers with ineffective had a high presence of *Aedes* immatures (3.69 and 2.54 times, respectively). In addition, the chemical properties of the water inside the containers, such as pH, influenced the presence of *Aedes* immatures (1.76 times) (OR = 1.76, 95% CI = 1.59–1.96).

Originality/value – The study results emphasized the characteristics of water containers in households located in a tourist attraction area. The findings may inform public health vector-control messages for households located in the tourist attraction area.

Keywords *Aedes* mosquito immature, Dengue vector, Mosquito survey, Tourist attraction area, Water container

Paper type Research paper

Background

In Thailand, the dengue virus is transmitted by *Aedes* mosquitoes, especially *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus*. These species prefer to breed in man-made water containers in human habitation. The risk of dengue transmission is increased by poor environmental management and water storage practices. The annual report of the World Health Organization (WHO) estimated that the global incidence of dengue, mostly among

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children, has increased significantly in recent decades[1]. For example, dengue cases in three WHO regions (Americas, South-East Asia and Western Pacific) exceeded 1.2m cases in 2008, and over 3m in 2013. Worryingly, the number of dengue cases is increasingly reported in new areas, and extensive dengue outbreaks have also occurred[2].

Dengue infection is prevalent in several endemic areas, including tourist attraction areas. Travelers infected in such areas can carry the dengue virus to their home country or other regions[3]. One study revealed that international travel was a factor related to dengue transmission[2]. In Japan, approximately 200 imported cases of dengue resulted from travel in South-East Asia[4, 5]. Tourist attraction areas may be important key/silent areas for dengue transmission. This study was conducted in Bang Kachao Riverbend, a famous eco-touristic bicycle travel park in Thailand. In 2015, the dengue incidence rate (165.80 per 100,000 population) in Bang Kachao Riverbend rose 3.5-fold compared with the incidence rate (47.37 per 100,000 population) in 2014[6]. More recently, dengue cases have occurred in Bang Kachao Riverbend every year.

Tourist attraction areas may provide breeding habitats for mosquitoes and raise the reproduction rate/prevalence of infective mosquitoes. In Thailand, most tourist attraction areas are closely co-located to areas of human habitation and related to household water containers. Therefore, water containers in households located in tourist attraction areas should be a matter for consideration.

The characteristics of water containers, such as the covering lids, their location (indoors or outdoors), container material, container type and the chemical properties of the water, affect the presence and abundance of *Aedes* mosquito larvae. Several studies have revealed that water container covers reduce mosquito oviposition[7, 8]. The location of water containers may be important in the site selection for mosquito oviposition. A study in Northern Queensland, Australia, found that more female *Ae. aegypti* mosquitoes were captured in outdoor ovitraps than indoor ovitraps[9]. *Aedes* mosquitoes prefer different water containers for oviposition, such as earthen jars, plastic bowls, flower pots, tires and disposed items. These containers are also made of different materials, i.e. plastic, rubber, cement, glass, natural materials and others. A study revealed that the duration of mosquito larva development varied in different container materials[10]. Another study found that the number of *Aedes* immatures per container was not distributed consistently in each water container[11]. This indicated that specific types of containers affected the selection of oviposition by female mosquitoes. The chemical properties of the water in the containers, including pH, total dissolved solids (TDS, mg/l), electric conductivity (EC, μS/cm) and dissolved oxygen (DO, mg/l), also affected the oviposition behavior of female mosquitoes [12]. The effects of pH on the acclimation of *Aedes* larval growth and development permit a low percentage of larvae to transform to the pupal stage successfully and increase the survival rate at extreme pH values[12]. TDS is a measure of the combined content of all inorganic and organic substances in a liquid in molecular, ionized or micro-granular suspended form. These inorganic salts and small amounts of organic matter are dissolved in water. The primary sources for TDS in receiving waters are agricultural and residential runoffs, clay-rich mountain waters or leaching of soil contamination. This type of water in containers may contain these particles and provide food and nutrients for developing mosquito larvae[12]. EC is also seen to play a major role in the oviposition preferences of larval mosquitoes. Several EC studies with a range of 162.9–1,656.8 μS/cm affected larval abundance[13]. However, if EC values are $> 2,000$ μS/cm, larval density is significantly reduced[14]. DO is important for several species of larval mosquitoes[12] and other aquatic invertebrates[15]. A study of urban rivers proximate to larval habitats in Chinese urban areas found that DO in breeding containers was negatively associated with overall larval abundance[16]. Another study in central Colombia found DO significantly affected *Ae. aegypti* larvae, with a range of 5.85–6.25 mg/l[17]. However, DO can vary daily and seasonally by as much as 1–20 mg/l, due to variations in natural processes, such as aeration, photosynthesis, diffusion, respiration, decomposition, temperature and air pressure[18].

This study aimed to investigate the characteristics of water containers influencing the presence or absence of *Aedes* immatures in households located in a tourist attraction area.

Methods

Study design and study area

A cross-sectional study was conducted in a tourist attraction area in Bang Kachao Riverbend, Phra Pradaeng District, Samut Prakan Province, Thailand (Figure 1) from October 2016 to September 2017. Bang Kachao Riverbend has an annual mean ambient temperature of 29.70–30.54°C, a relative humidity of 64.30–70.86 percent and rainfall of 265.55 mm. The area has been developed for international ecotourism since 2002. Since 2015, the dengue incidence rate here has increased to about 165.80 per 100,000 of the inhabitant population. It has risen 3.5 times the incidence rate (47.37 per 100,000 population) in 2014[6]. Nowadays, dengue cases in Bang Kachao Riverbend occur every year.

This study was targeted to collect data in households located in the study area. The studied households were estimated using the formula $n = (Np(1-p)Z^2/(d^2(N-1)+p(1-p)Z^2))$[19] with N being number of households (448) and $p = 0.59$[20]. The minimum sample size was 204 households per data collection. In total, 204 households were selected by systematic random sampling method in the research area by selecting households with reported recent dengue experience, and its neighboring houses, making up two houses adjacent to the first house.

Data collection

Study data were collected four times: October to December 2016, January to March 2017, April to June 2017 and July to September 2017. Before each household survey, a village health volunteer (VHV) was trained as a research assistant to assist in data collection for each team (total of three teams). For the cross-sectional field surveys, 832 households were

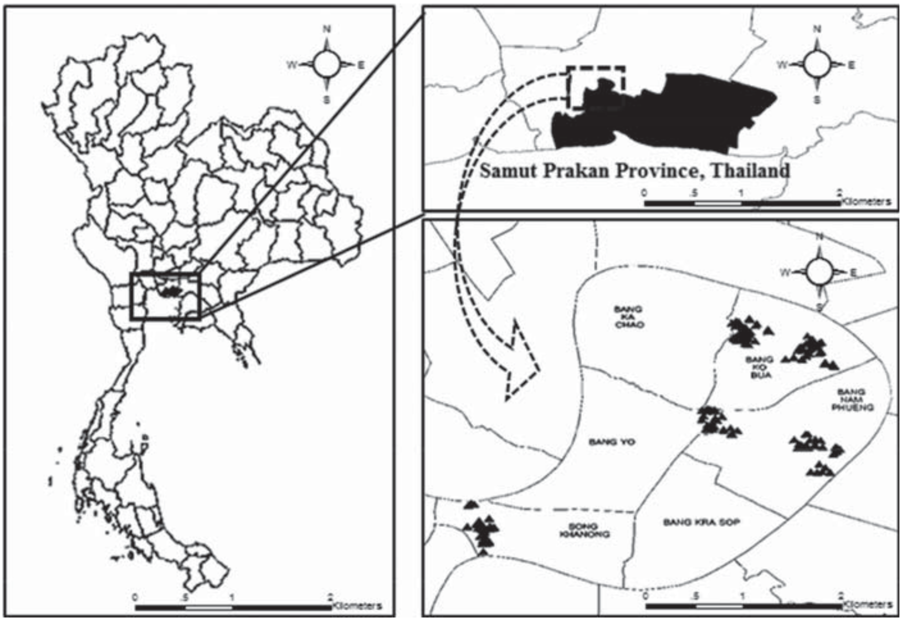

Figure 1.
Location of studied households (black triangles) in Bang Kachao Riverbend, Samut Prakan Province, Thailand

selected randomly from five villages: village 2 Bang Kobua, village 10 Bang Yo, village 3 Bang Nam Phueng, village 8 Bang Kobua and village 9 Song Kanong. Permission to collect data from the participants in each household was requested by a VHV. An informed consent form was provided for participant signature. The research objectives and details were explained to each participant. Interviews were conducted with the head of the household or representative using a structured questionnaire. At the same time, two research assistants inspected indoor and outdoor water containers for mosquito larvae. The household was defined into two categories: a household where we found *Aedes* immatures mosquito was recorded as "the presence of *Aedes* mosquito immatures" or $Y = 1$, and a household with no trace of *Aedes* immatures mosquito was recorded as "the Absence of *Aedes* mosquito immatures" or $Y = 0$.

For the *Aedes* larvae/pupae collections, in the case of small containers, larvae/pupae were directly collected by emptying those containers into a tray with a filtered sieve. A pipet was used to remove larvae/pupae from a filtered sieve to a labeled 120 mL plastic bottle (about 20 larvae/pupae per a bottle) with water (keeping an air space of at least 1 cm). In the case of a large container with low densities of larvae/pupae (< 100), a comprehensive netting was used to carefully immerse 7.5 cm beneath the water surface of the containers and move around the border in a descending spiral. Larvae/pupae, moved at the bottom center of the containers, were then scooped up in the net and then collected as previously described[21]. If there were high densities of larvae/pupae (> 100), they were collected by 1/3 CF (Calibration Factor) or 1/3 full based on the volume of water present in the container[21]. For each water container, pH, TDS, mg/l; electrical conductivity (EC, μS/cm); and DO, mg/l were measured using an HQ30D portable multi-meter (HACH Company, Model No. HQ30D53000000).

All details (i.e. location, date, time and type of container) were recorded using the household *Aedes* larval survey form that had been developed. After that, the samples were transported to the laboratory at the Medical Entomology Insectarium, Faculty of Tropical Medicine, Mahidol University, in Bangkok. All samples were counted and the number recorded. *Aedes* larvae were kept in plastic micro-tubes with absolute ethanol for taxonomic identification of the mosquito species.

Identification of Aedes larvae and adults

All *Aedes* larvae were identified by the presence of the comb scale on the terminal segment. The comb scale of *Aedes aegypti* is a single row with pitch-fork shape, whereas the comb scale of *Aedes albopictus* is a single row with straight thorn-like shape[22]. All *Aedes* pupae were reared to the adult stage before identification. These *Aedes* adults were identified by the presence of patterns on the dorsal surface of the thorax. *Ae. aegypti* presents thoracic patterns with black scales and typical lyre-shaped silvery markings on the lateral edges of the scutum, whereas *Ae. albopictus* displays thoracic patterns with a prominent silvery central line[23].

Categorization of containers

All water containers under observation were categorized into 17 groups: bathroom and toilet; daily used water; ant plates; pet water bowls; water storage containers for use when daily water sources were lacking; flower vases; temporary containers; receptacles for fallen or leaking water, Gutters; drinking water; containers with immersed materials that always stored water; water bowls or glass containers in cemeteries and spirit houses; bins with discarded/recycled refuse; basins for aquatic plants; flower pots; water retained in plant leaf axils and tree-holes; tires; and lids, flipped bottoms and edges of containers. The results of all positive containers are shown in Table I. The characteristics of water containers and water quality were classified as follows: location (indoors or outdoors); container material (plastic/rubber, cement, natural habitat); cover or lid on the water container (none,

Containers	Indoors *n*	%	Outdoors *n*	%	Total *n*	%
1. Bathroom and toilet	151	74.02	27	6.72	178	29.37
2. Daily use water	21	10.29	71	17.66	92	15.18
3. Ant plates	15	7.35	1	0.25	16	2.64
4. Pet water bowls	4	1.96	7	1.74	11	1.82
5. Storage container; to use when daily use water is lacking	3	1.47	13	3.23	16	2.64
6. Flower vases	3	1.47	10	2.49	13	2.15
7. Temporary containers	2	0.98	20	4.98	22	3.63
8. Receptacles for fallen/leaking water, Gutters	2	0.98	7	1.74	9	1.49
9. Drinking water	1	0.49	0	0.00	1	0.17
10. Containers of immersed materials used to store water	1	0.49	4	1.00	5	0.83
11. Water bowls or glass containers in cemeteries and spirit houses	1	0.49	20	4.98	21	3.47
12. Bins for discarded/recycled refuse	0	0.00	65	16.17	65	10.73
13. Basins for aquatic plants	0	0.00	12	2.99	12	1.98
14. Flower pots	0	0.00	54	13.43	54	8.91
15. Water retained in plant leaf axils and tree-holes	0	0.00	54	13.43	54	8.91
16. Tires	0	0.00	10	2.49	10	1.65
17. Lids, Flipped bottoms and edges of containers	0	0.00	27	6.72	27	4.46
Total	204	100.00	402	100.00	606	100.00

Table I. Number and percentage of positive water containers, by location (indoors and outdoors)

ineffective lid[a], effective lid[b]); the chemical properties of the water in the container (pH; TDS, mg/l; EC, μS/cm; and DO, mg/l).

Notes: [a], [b]An "effective lid" in this study means a lid that tightly covers a water container. It prevents both mosquito entry to lay eggs and the escape of newly emerged mosquitoes; an "ineffective lid" cannot prevent the entry/egress of mosquitoes due to the gap between the lid and the edge of the water containers.

Data analysis

STATA® version 14 was used for data analysis. Number and percentage were used to describe the characteristics of the water containers. The factors of the characteristics of the water containers that influenced the presence of *Aedes* immatures were analyzed using a binary logistic regression model, reporting odds ratios.

Ethical consideration

This study was approved by the Tropical Medicine Ethics Committee, Faculty of Tropical Medicine, Mahidol University (MUTM 2016-068-01) with an approval date of September 19, 2016. Permission for animal care and use of *Aedes aegypti* mosquitoes was approved by Mahidol University-Institute Animal Care and Use Committee, Mahidol University (MU-IACUC 2016/023) with an approval date of September 19, 2016.

Results

Mosquito productivity

A total of 46,077 *Aedes* immatures (pupae and larvae) were collected from 318 positive households (38.22 percent of 832 households). *Ae. aegypti* and *Ae. albopictus* were identified at 99.23 and 0.72 percent, respectively. Among the positive water containers, the average numbers of *Aedes* immatures were 133.1 immatures/container inside households and 47.1 immatures/container outside households. Among the positive containers (total = 606 containers, indoor = 204 containers, outdoor = 402 containers), bathroom and toilet containers were the most common (29.37 percent), followed by water-for-daily-use containers (15.18 percent), bins for discarded/recycled refuse (7.41 percent), flower pots, and water retained in plant leaf axils and tree-holes (8.91 percent) (Table I). Regarding the location of the positive containers, the study found that the proportion of indoor positive containers was high in bathrooms and toilets (Figure 2).

Characteristics of water containers

About 72 percent of water containers were located outdoors and 49 percent were made of cement; ~56 percent were not covered with lids, while only 19.6 percent were covered with effective lids (Table II).

The water quality in all containers is very important to mosquito development. They affect larvae/pupae development and also affect the ovipositional behavior of female mosquitoes. This study showed an average pH of 7.92±0.36 indoors and 7.79±0.54 outdoors, average TDS of 217.49±644.70 mg/l indoors and 177.75±387.90 mg/l outdoors, average EC of 434.32±1289.53 μS/cm indoors and 355.05±776.10 μS/cm outdoors, an average DO of 4.60 ±0.71 mg/l indoors and 5.46±19.31 mg/l outdoors (Table III).

Factors influencing the presence of Aedes immatures

The study found that the water containers located inside households (indoors) had a high presence of *Aedes* immatures (2.22 times) compared with the outdoor containers (OR = 2.22, 95% CI = 2.18–2.26). Water containers without lids (OR = 3.69, 95% CI = 1.91–7.13) and water containers with ineffective lids (OR = 2.54, 95% CI = 1.20–5.38) showed a highly presence of *Aedes* immatures (3.69 and 2.54 times, respectively). In addition, the chemical properties of water in the containers, such as the pH, influenced the presence of *Aedes* immatures (1.76 times) (OR = 1.76, 95% CI = 1.59–1.96). However, this study did not find

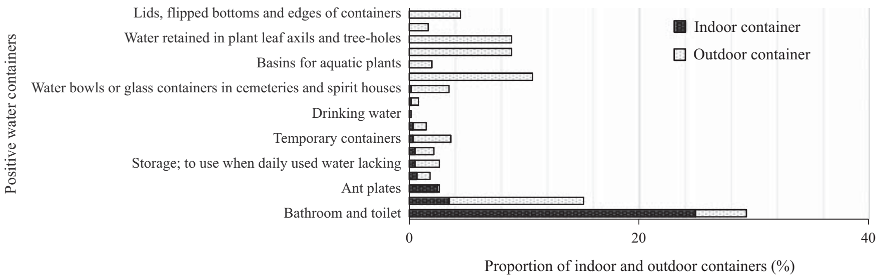

Figure 2. Proportion of indoor and outdoor containers

Independent variables	Number	%
Location of container		
Indoors	234	28.12
Outdoors	601	71.88
Material of container		
Plastic/or rubber	244	29.33
Cement	410	49.27
Natural habitat	178	21.4
Cover or lid status		
None	473	56.85
Ineffective lid	196	23.56
Effective lid	163	19.59

Note: $n = 832$

Table II. Characteristics of the water containers any significant effect for container material or chemical properties of the water in the containers, such as TDS, EC and DO, on the presence of *Aedes* immature production ($p > 0.05$) (Table IV).

Discussion

The differences for productive containers bathroom and toilet containers (74.02 and 15.17 percent), and daily-use water containers (10.29 and 77.17 percent) were the indoors and outdoors locations, respectively. Both consistently provided breeding places for *Aedes'* immatures production. The results were similar to previous studies finding that open-mouthed containers and large containers increased the number of suitable breeding places for mosquito larval production[24, 25]. Indoors, large or open-mouthed containers in the bathroom and toilet, which are human-made square concrete basins, were the most productive sources of *Aedes* immatures. Outdoors, daily-use water containers for washing clothes or cleaning the house, mostly using earthen jars, were the most productive. Bang Kachao Riverbend is an environmental area that provides breeding places that support abundant *Aedes* immatures. These breeding areas include natural water held in natural containers, such as tree-holes, bromeliad leaf axils and bamboo trunks, which are difficult to eliminate and should be considered for priority primary control.

Table III. Chemical properties of the water inside the containers

Independent variables	Mean ±SD Indoors	Outdoors
pH	7.92 ± 0.36	7.79 ± 0.54
Total dissolved solids, TDS (mg/l)	217.49 ± 644.70^a	177.75 ± 387.90^a
Electrical conductivity, EC (μS/cm)	$434.32 \pm 1,289.53^b$	355.05 ± 776.10^b
Dissolved oxygen (DO, mg/l)	4.60 ± 0.71	5.46 ± 19.31

Notes: [a]Indoor and outdoor: minimum TDS = 7 mg/l, maximum TDS = 9,122 mg/l; [b]indoor and outdoor: minimum EC = 14 μS/cm, maximum EC = 18,244 μS/cm

Table IV. Binary logistic regression analysis of characteristic factors of water containers influencing the presence of *Aedes* mosquito immatures

Independent variables	OR	95% CI	*p*-value
Location of container			
Indoors	2.22	2.18–2.26	< 0.001*
Outdoors (reference)	1.00		
Material of container			
Plastic/or rubber	1.15	0.69–1.91	0.598
Cement/or glass	1.29	0.89–1.86	0.182
Natural habitat (reference)	1.00		
Cover or lid status			
None	3.69	1.91–7.13	< 0.001*
Ineffective lid	2.54	1.20–5.38	0.014*
Effective lid (reference)	1.00		
Chemical properties of the water in the containers			
pH	1.76	1.59–1.96	< 0.001*
Total dissolved solids, TDS (mg/l)	1.00	0.99–1.01	0.314
Electrical conductivity, EC (μS/cm)	1.00	0.99–1.01	0.290
Dissolved oxygen (DO, mg/l)	0.85	0.66–1.08	0.179

Notes: $n = 832$ households. *$p < 0.05$

An important characteristic factor for water containers influencing the presence of *Aedes* immatures production was the location of the water container (Table IV). Water containers located inside households (indoors) had a higher presence of *Aedes* immatures than outdoor containers. This result may be supported by the observation that this is a dengue-endemic area. The people in Bang Kachao Riverbend mostly use large and open-mouthed containers, which are handmade and normally used to store water inside households, such as a square concrete basin in the bathroom (74.02 percent of the locations where *Aedes* immatures were found). A square concrete basin for the bathroom is larger than an earthen jar or a plastic bucket, and it also contains a large volume of water. Water in this container is periodically refilled. Moreover, earthen jars and plastic buckets without lids or with ineffective lids used for storing water for daily use (10.29 percent) are also often installed inside households. As mentioned above, these containers and usage practices might be key causes of dengue virus transmission in this area. The results show that vector control in Bang Kachao Riverbend should focus on productive indoor containers; however, outdoor containers should not be ignored.

The study also found that cover or lid status influenced the presence of *Aedes* immatures. Water containers without lids were found to have a significantly higher presence of *Aedes* immatures. This result was similar to previous studies[26, 27]. Indoor and outdoor water containers with inadequate covers are suitable containers in which female mosquitoes can lay their eggs. Containers covered by lids may not be a complete shield if they are not covered with tightly fitting lids. Covering lids are recommended by the WHO for effective, low-cost vector control. However, square concrete basins without covers in bathrooms have formed part of the traditional lifestyle of the local people in this area, so another appropriate method of mosquito control should be applied.

The chemical properties of the water in containers are important for the development of the immature stage and also affect the oviposition behavior of female mosquitoes[17]. The study found that pH levels influenced the presence of *Aedes* immatures. The average pH of the indoor and outdoor containers was about 7.92 and 7.79. In India, water pH had a significant correlation with larval density in container-breeding mosquitoes[28]. Another study implied that a pH range of 6.5–8.0 is suitable for *Aedes aegypti* survival[29]. Among various Thai traditional cultures, "red lime," a material for chewing with betel nut and betel leaf, could be used to increase the pH value/alkalize the water in containers[30]. Spraying a bio-pesticide, such as neem (*Azadirachta indica*) oil, could also be used to manage pH, as practiced in India[31].

Conclusions

These results might be supported by the public health concern in the households that are located in a tourist attraction area. Different productive containers were often found with *Ae. aegypti* pupae representing more than 70 percent of surveillance, such as in jars, toilet tanks, fish bowls and plastic pans surveyed in Khon Kaen province (northeastern Thailand), Chiang Mai province (northern Thailand) and Surat Thani province (south of the country) during rainy and dry seasons. However, the pH or other substances in containers should be evaluated in further studies. Using safe and environmentally friendly water storage systems are recommended as well as increasing the knowledge of humans and society about the dangers of certain practices. The increase of *Aedes* immatures is a public health concern and is mainly achieved by eliminating container habitats that are favorable in oviposition sites. These controls may not permit the development of the larvae/pupae stages. For daily use, containers are able to eliminate larvae/pupae by cleaning them or removing the developing stages using an insecticide or biological control agent or combination of these methods. Finally, applying an integrated vector management system is the strategic approach to promoting vector control. These findings are an early warning to control dengue vector in a tourist attraction area.

Acknowledgments

The authors declare that there is no conflict of interest.

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