DIFFERENCE IN LARVAL HABITAT SIZE BETWEEN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN A DENGUE-ENDEMIC VILLAGE, LAO PEOPLE'S DEMOCRATIC REPUBLIC

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Abstract. The larval habitats of *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) in a dengue-endemic rural village in Khammouane Province, Lao People's Democratic Republic, as well as potential factors (water volume, type of container, water source, water color, sunlight, and presence of aquatic organisms other than mosquitoes) associated with the presence of mosquitoes were analyzed. *Aedes aegypti* was most frequently found in water storage jars, while *Ae. albopictus* infested various water-holding containers, such as jars, used tires and discarded waste containers. Logistic regression analysis showed that prevalence of *Ae. aegypti* was higher in water storage jars than in other containers, while that of *Ae. albopictus* was higher in small water containers. Median water volume infested with *Ae. albopictus* was 1 liter, significantly smaller than median of 50 liters for infestation with *Ae. aegypti*. On the other hand, prevalence of both mosquito species was not associated with water source, water color, sunlight, or presence of aquatic organisms other than mosquitoes. Thus, larval habitat size and type differed between *Ae. aegypti* and *Ae. albopictus*, revealing that different larval source management policies and tools are needed to be implemented to each target vector species to control dengue transmission.

Keywords: *Aedes aegypti*, *Aedes albopictus*, dengue, vector control, larval source management, Lao PDR

INTRODUCTION

Over the past 50 years, global incidence of dengue, a mosquito-borne viral disease, has increased 30 folds (WHO, 2012), with 70% of cases occurring in Asia (Bhatt *et al*, 2013). Lao People's Democratic Republic (PDR) has one of the highest incidence rates in Asia (Khampapongpane *et al*, 2014; Arima *et al*, 2015). The primary vector of dengue, *Aedes aegypti* (L.), inhabits human settlements in urban and peri-urban areas, while the secondary vector, *Ae. albopictus* (Skuse), is associated with peri-urban and rural environments (Braks *et al*, 2003; Tsuda *et al*, 2006). As there are no antiviral drugs or definitive vaccines for dengue, vector control is vital to pre-

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vent dengue outbreaks and transmissions (WHO, 2017a).

A sustainable vector control in dengue-endemic countries is larval source management, such as fitting covers on containers and eliminating larvae using insecticides or biological control agents (WHO, 2009). *Ae. aegypti* and *Ae. albopictus* are known to breed in artificial containers, such as water storage jars, drums, discarded used tires, and cement tanks, although the species exhibit varying preferences for different types of containers (Chan *et al*, 1971; Ishak *et al*, 1997; Phong and Nam, 1999; Tsuda *et al*, 2002; Chareonviriyaphap *et al*, 2003). In addition, the key breeding containers utilized greatly differ among endemic countries (Focks and Alexander, 2006; Tun-Lin *et al*, 2009; Arunachalam *et al*, 2010).

In order to achieve a cost-effective larval source management, it is essential to understand which type(s) of container is (are) the most productive habitat(s) for each vector mosquito species within a given area. The present study characterized larval habitats of *Ae. aegypti* and *Ae. albopictus* by surveying a dengue-endemic rural village in Khammouane Province, Lao PDR and analyzing the ecological factors associated with mosquito presence. On the basis of larval habitat differences, appropriate control methods for *Ae. aegypti* and *Ae. albopictus* are discussed.

MATERIALS AND METHODS

Study site

This study was conducted in a rural village, Nyang Khao (17°18′20.07″N, 104°55′2.33″E) consisting of 120 households and 679 inhabitants in 2013, in Thakhek District, Khammouane Province, Lao PDR. The village is occupied by farmers and surrounded by agricultural paddy fields.

Larval survey

Larval survey was conducted in September (during the rainy season) 2013. Forty households were randomly visited and, with permission of the residents, all possible mosquito-breeding habitats within the premises were sampled. Water storage jars, plastic drums and buckets, used tires, discarded wastes, cement tanks, iron drums, and natural habitats on plants were inspected for the presence of immature mosquitoes. The most common type of container in the village was a 150–200-liter earthen jar. Mosquito larvae and pupae in large containers such as jars and plastic drums were collected by netting (sweeping the net five times per each container), and water within small containers (capacity < 5 liters) by completely pouring into a white tray (30 × 21 × 5 cm). The presence of third- and fourth-instar larvae and/or pupae of *Aedes* and *Culex* spp in each container was recorded, along with the approximate water volume (liter) within the container, type of container, water source (rain or well), water color (clear or cloudy/ dirty), presence of sunlight (sun or shade), and presence of aquatic organisms other than mosquitoes. More than five larvae and all pupae of genus *Aedes* were kept in 50-ml tubes and brought to the laboratory for identification. Larvae were mounted on slides and identified according to the keys provided by Rattanarithikul *et al* (2010). Pupae were identified based on adult morphology after emergence. Records of both larvae and pupae were included in the analysis.

Data analysis

Multivariate logistic regression analysis was employed to determine the key factors accounting for the presence or absence of immature *Ae. aegypti* and *Ae. albopictus* in water-holding container. All parameters recorded for each container inspected were included in the logistic model. Subsequently, prevalence of each species in water storage jars and other containers was compared using χ^2 test. Differences in containers' water volume were compared between *Ae. aegypti* and *Ae. albopictus* based on Mann–Whitney *U* test. Statistical analysis was performed in JMP 11.2.1 (SAS Institute Japan, Tokyo, Japan), with significance at a *p*-value <0.05.

RESULTS

Type of containers infested by mosquitoes

Of 106 water-holding containers investigated, 14%, 14%, and 23% were infested with larvae and/or pupae of *Ae. aegypti*, *Ae. albopictus*, and *Culex* spp, respectively. *Ae. aegypti* was most frequently found in earthen jars, followed by discarded used tires, while *Ae. albopictus* infested earthen jars, used tires, sources of discarded waste water, and unused small jars (Fig 1). *Culex* mosquitoes were found in a wide range of containers, such as earthen jars, used tires, waste water, buckets, unused small jars, and axils of plant leaf (Fig 1).

Factors affecting presence of *Ae. aegypti* and *Ae. albopictus* immatures

Although the presence of *Ae. aegypti* was not associated with water volume [odds ratio (OR) = 1.00, 95% confidence interval (CI): 0.99-1.01, p = 0.9991], the presence of *Ae. albopictus* significantly decreased by 2% with each 1-liter increase in water volume (OR = 0.98; 95% CI: 0.95-1.00, p = 0.0285; Table 1). The type of container was an important factor affect-

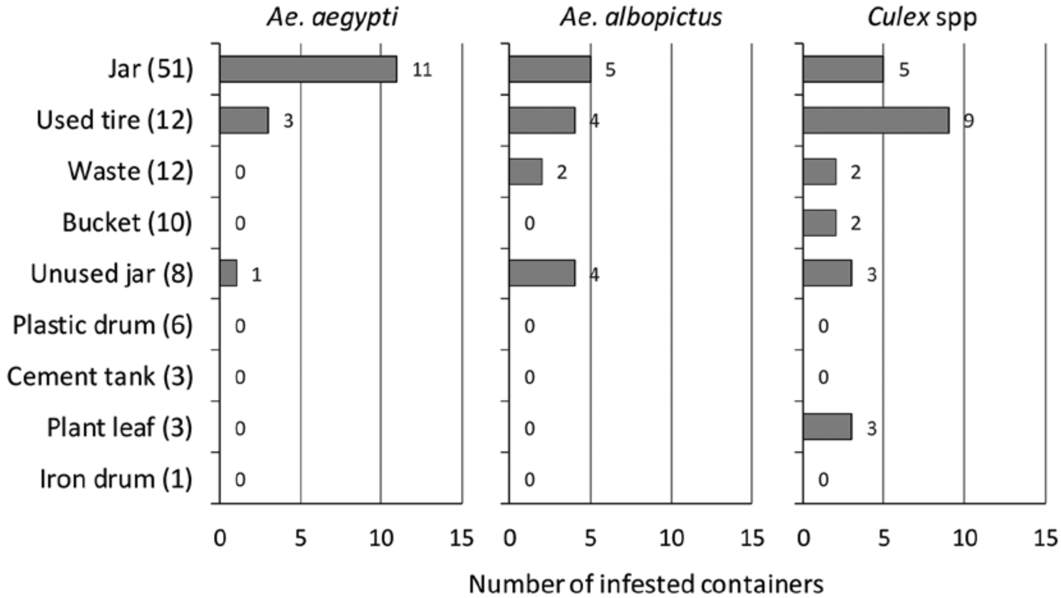

Fig 1-Number of containers infested with *Aedes aegypti*, *Ae. albopictus*, and *Culex* spp in Nyang Khao Village, Thakhek District, Khammouane Province, Lao PDR in September, 2013. Number in parenthesis indicates the sample size for each type of container.

Table 1
Multivariate logistic regression analysis of potential factors affecting presence of immature stages of *Aedes aegypti* and *Ae. albopictus* in water-holding containers in Nyang Khao Village, Thakhek District, Khammouane Province, Lao PDR in September, 2013.

Factor	*Ae. aegypti*		*Ae. albopictus*	
	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
Water volume (liter)				
0.01~300	1.00 (0.99-1.01)	0.9991	0.98 (0.95-1.00)	0.0285
Type of container				
Other containers	1		1	
Jars	5.76 (1.24-33.71)	0.0247	1.95 (0.35-11.19)	0.4435
Source of water				
Well water	1		1	
Rain water	2.86 (0.76-12.55)	0.1238	2.3 (0.44-17.64)	0.3331
Water color				
Cloudy or dirty	1		1	
Clear	0.98 (0.22-5.18)	0.9759	1.05 (0.27-4.35)	0.9495
Sunlight				
Sun	1		1	
Shade	1.626 (0.48-5.95)	0.4375	2.04 (0.61-7.78)	0.2539
Other aquatic organisms				
Absent	1		1	
Present	1.68 (0.43-6.12)	0.4413	1.45 (0.34-5.45)	0.5925

CI, confidence interval.

ing the presence of *Ae. aegypti*. *Ae. aegypti* immatures infested jars 5.76 times more frequently than other containers (OR = 5.76; 95% CI: 1.24-33.71, p = 0.0247; Table 1). However, infestation by *Ae. albopictus* was not significantly associated with the type of container (OR = 1.95; 95% CI: 0.35-11.19, p = 0.4435; Table 1). Water source and color, and presence of sunlight and of other aquatic organisms did not significantly affect the presence of *Ae. aegypti* or *Ae. albopictus* (Table 1).

A comparison between water volume of containers infested by the two *Aedes* spp revealed a significant difference (Z-value = 2.89, p = 0.0038; Fig 2). *Ae. aegypti* immatures infested containers with a wide range of water volume (median = 50 liters, range = 0.5-150 liters), whereas *Ae. albopictus* immatures tended to inhabit small water containers (median = 1 liter, range = 0.1-80 liters).

The prevalence of *Ae. aegypti* in jars was 22%, significantly higher than in other types of containers (χ^2 = 4.6, df = 1, p = 0.0324; Fig 3). *Ae. albopictus* was more

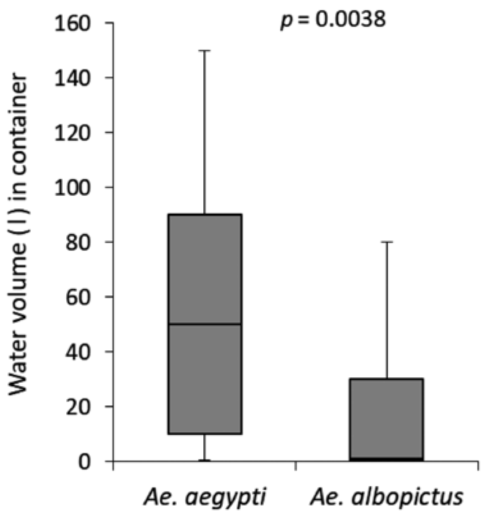

Fig 2-Water volume (liter) in the containers infested with *Aedes aegypti* (n = 15) and *Ae. albopictus* (n = 15) in Nyang Khao Village, Thakhek District, Khammouane Province, Lao PDR in September 2013. Horizontal line within box indicates median value; upper and lower borders of the boxes indicate the 75th and 25th percentile, respectively; and vertical line represents minimum and the maximum values. Values were compared using Mann–Whitney *U* test.

frequently found in other containers than jars, although this difference was not significant ($\chi^2 = 1.6$, df = 1, $p = 0.2118$; Fig 3).

DISCUSSION

Water storage jars were the most important breeding site for *Ae. aegypti*, followed by discarded used tires, although other water containers were available. On the other hand, *Ae. albopictus* immatures were distributed in small habitats, such as discarded waste water sources and unused containers. These results were consistent with those in Thailand of Chareonviriyaphap *et al* (2003), who reported *Ae. aegypti* prefers to breed in water storage jars, whereas *Ae. albopictus* inhabits various water sources such as discarded cans and tires. Moreover, our results showed that the median water volume infested with *Ae. albopictus* was much smaller than that infested with *Ae. aegypti*, emphasizing the difference in habitat size of *Ae. aegypti* and *Ae. albopictus* in Nyang Khao, Thakhek District, Lao PDR.

We did not investigate indoor containers, except for the toilet; therefore, our results may be limited in scope. *Ae. aegypti* is known to inhabit small indoor containers such as ant traps and flower vases (Chan *et al*, 1971; Tsuzuki *et al*, 2009), which is not consistent with our findings. Nevertheless, other studies in Lao PDR have shown a high infestation of *Ae. aegypti* in water storage containers, such as water storage jars, drums and water tanks (Tsuda *et al*, 2002; Hiscox *et al*, 2013), as well as in other areas of Asia (Lee, 1991; Phong and Nam, 1999; Chareonviriyaphap *et al*, 2003; Tsuzuki *et al*, 2009; Arunachalam *et al*, 2010). Thus, to control *Ae. aegypti* in Lao PDR, it is important to prevent breeding in water storage containers.

A multi-country study revealed targeted control of breeding water sources leads to cost-effective reduction in vector density compared with non-targeted control (Tun-Lin *et al*, 2009). As water storage jars were the most productive water containers for *Ae. aegypti* breeding in this study area, targeted control exerted on this type of container should contribute to a large reduction in *Ae. aegypti* population. However, it is difficult to eliminate water storage jars from households in this region of Asia, even when tap water is available (Nguyen *et al*, 2011). Both prevention and control measures should be employed to control breeding of mosquitoes in these containers. For example, mosquito breed-

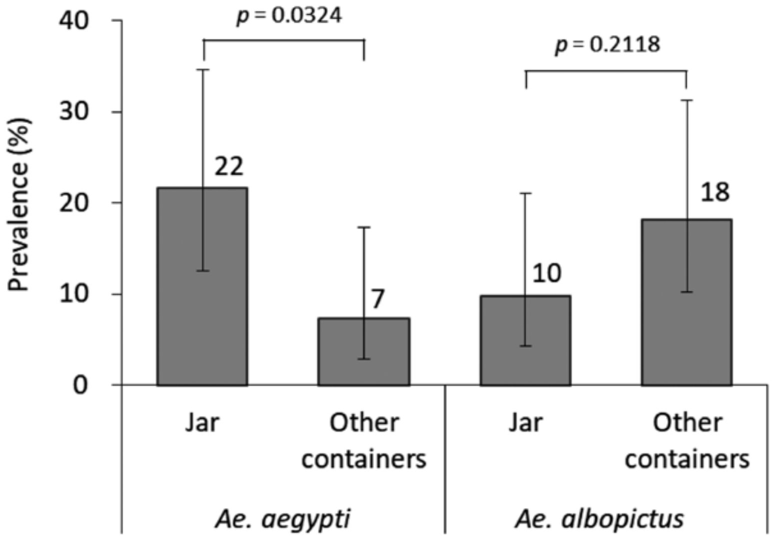

Fig 3-Prevalence (±95% CI) of *Aedes aegypti* and *Ae. albopictus* in water jars and other containers in Nyang Khao Village, Thakhek District, Khammouane Province, Lao PDR in September 2013. Prevalence of each species was compared using the χ^2 test.

ing could be prevented by fitting covers on containers (Tsuzuki *et al*, 2009) and/or using insecticide-treated nets to cover the mouths of such containers (Kroeger *et al*, 2006; Vanlerberghe *et al*, 2011; Tsunoda *et al*, 2013). Application of larvicides or biological control agents to storage water should also be considered. Insecticides, such as organophosphates, insect growth regulators and biopesticides are effective in controlling mosquito larvae (WHO, 2017b). For example, matrix-release formulations of the insect growth regulator pyriproxyfen have been shown to provide control for at least six months in mosquito-breeding containers in Cambodia (Seng *et al*, 2008), Malaysia (Ohashi and Shono, 2015), and Japan (Ohashi, 2017). Furthermore, long-lasting formulations reduce operational cost per year of treatment, making sustainable control of *Ae. aegypti* more affordable.

Both *Aedes* species utilized discarded used tires as breeding sites and often co-inhabited these sites, as documented in other studies (Suwonkerd *et al*, 1997; Higa *et al*, 2010; Gautam *et al*, 2015). Hence, used tires should be removed to eliminate a potential breeding site. Alternatively, tires could be used as lethal oviposition traps because gravid *Aedes* females are attracted to water that is already inhabited by conspecific larvae (Wong *et al*, 2011; Gonzalez *et al*, 2016). An insect growth regulator such as pyriproxyfen, which kills pupae but not larvae, can maintain the attractiveness of such sites for gravid females and therefore has great promise as a control agent (Wong *et al*, 2011).

Aedes albopictus might be more difficult to control than *Ae. aegypti* due to its wider range habitats, such as discarded wastes and unused containers. Hiscox *et al* (2013) previously reported that in Lao PDR *Ae. albopictus* was 3.64 times more frequent in discarded waste water than in storage containers. Therefore, eliminating water wastes and keeping dry containers that are not in use are key factors in reducing *Ae. albopictus* infestation. In addition, using small lethal oviposition traps could help prevent the remaining *Ae. albopictus* population from breeding.

Ohba *et al* (2013) demonstrated that pyriproxyfen-treated nets suppress *Ae. albopictus* population. At high dose, females that rested on treated nets become completely sterilized and their eggs did not hatch, and at lower dose, the chemical was transferred from the treated nets to breeding sites by gravid females and produced high rates of pupal mortality. Although the use of pyriproxyfen-treated materials might potentially sterilize adult *Aedes* mosquitoes, their susceptibility to pyriproxyfen (Ohba *et al*, 2013) is lower than that of *Anopheles* (Ohashi *et al*, 2012). The auto-dissemination approach using gravid females as a vehicle to transfer the larvicide to their breeding sites may help reduce *Ae. albopictus* population inhabiting small inaccessible habitats (Itoh, 1995; Devine *et al*, 2009; Caputo *et al*, 2012; Unlu *et al*, 2017).

In conclusion, our results showed *Ae. aegypti* and *Ae. albopictus* larval habitats differ in sizes and types, and thus different control tools will be required for each species to control dengue transmission.

ACKNOWLEDGEMENTS

This study was conducted with the cooperation of the Department of Public Health, Kansai Medical University, Osaka, Japan; the Health and Crop Sciences Research Laboratory, Sumitomo Chemical Co., Ltd., Hyogo, Japan; and Khammouane Provincial Health Department, Khammouane, Lao PDR. The authors thank Dr Nao Boutta and staff members in Cabinet, Ministry of Health, Lao PDR for their support of this study, Dr Somsanouk Arounleuth and staff members in the Provincial Malariology, Parasitology and Entomology Office, Khammouane, Lao PDR for implementation of the larval survey in the village, and Mr Issaku Nakatani, Mr Pheophet Lamaningao and Mr Phokhaysavanh Phothisane for support of the study.

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