ORIGINAL ARTICLES

Key breeding sites of Aedes mosquitoes in Huaylau village, Pakse city, Champasack province, Lao People Democratic Republic 2019

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ABSTRACT

Objectives: The elimination of key breeding sites of dengue vector is applied worldwide to control the vector density under the critical epidemic threshold. This study aimed to identify key breeding sites of Aedes mosquitos and associated factors in Huaylau village, Pakse city, Champasack province in Laos in 2019.

Methods: This was a cross-sectional study, combining of immature stage survey of Aedes mosquitoes in all water and discarded containers and the community's knowledge and practices on the prevention of dengue fever in all 240 households in Huaylau village. Multivariate logistic regression was applied to identify factors associated with the absence of Aedes larvae and pupae in the village. Statistical significance was set at a p-value less than 0.05.

Results: Among the 720 water and discarded containers surveyed, the study found 654 Aedes larvae and pupae; of which 94.6% were immature Aedes aegypti. Key breeding sites of Aedes aegypti included water jars (34.2%), plastic drums (30.7%), tanks < 500 litter (14.7%), and discarded containers (12.6%), while that of all Aedes albopictus was discarded containers. The community's better practices on the prevention of dengue fever contributed to the increased odds of the absence of Aedes larvae ad pupae by 1.612 (95%CI: 1.218-2.134, p<0.01), and the cleanliness of the house were associated with the increased odds of 4.072 of the absent Aedes larvae and pupae in the participated households (95%CI: 1.589-10.434, p<0.01).

Conclusions: Aedes aegypti mosquitoes in the Huaylau village laid their eggs in various breeding sites, including water containers and discarded containers while discarded containers were the only key breeding site of Aedes albopictus. Better practices on dengue prevention and better hygiene conditions of the houses contributed to the absence of Aedes larvae and pupae. Special attention should be paid to the clearance of all discarded containers and education of the community people to cover water containers in the village.

Keywords: *key breeding sites, Aedes, mosquito, pupae, larvae, Champasack province, Lao PDR.*

INTRODUCTION

Dengue fever (DF) is a rapidly spreading mosquito-borne viral disease and caused by four serotypes of virus belonging to the Flaviridae family, namely DENV-1, DENV-2, DENV-3, DENV-4, with distinct epidemiological patterns. The infection of one serotype may provide lifelong immunity to that type, but not confer protective immunity to the other three types, which means it is possible to be infected with DF more than one time (1). Dengue caused a wide range of clinical symptoms, from subclinical

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disease to severe flu-like symptoms, with the incubation period from 3 days to 14 days. Dengue imposes a heavy socio-economic and disease burden. Dengue outbreaks are observed mainly in the tropical and sub-tropical climatic regions, with approximately 400 million people from 128 countries living under the threat of dengue. Globally, there are more than 20,000 dengue-related deaths each year (2-5). According to The Global Burden of Disease, the increasing rate of dengue is higher than any other communicable disease, with a 400% increase from 2000 to 2013. Population growth, unplanned urban development, poor water storage and unsatisfactory sanitary conditions lead to the worsening burden of this mosquito-borne disease (6).

Aedes aegypti and, Aedes albopictus are the known vectors of dengue and other arboviral diseases including chikungunya, yellow fever and Zika (7, 8). They live mainly in the urban area and lay their eggs in both natural and artificial water containers near human habitation. After hatching from the egg within a few days to months, a larvae metamorphoses into a pupa, and subsequently into a terrestrial, flying adult mosquito. This metamorphosis stage of the mosquito's life is also aquatic (9). Aedes albopictus is well adapted in a broader temperature range and can survive in cooler temperatures than Aedes aegypti (10). The most productive containers for Aedes pupae are discarded plastic cups and bottles, flowerpots, drains, cement tanks, drums and used tires (1). In fact, there is no vaccine nor any specific medicine to treat the disease, and controlling mosquito vector populations is the best option available to prevent the dengue outbreak (11). The elimination of key breeding sites near human habitats has been considered as one of the most appropriate and effective approaches to manage this vector. The United States Agency for International Development (USAID) chose the "key container" survey conducted in Vietnam to improve dengue vector surveillance and vector control as one of the "best practices for environmental management of dengue" in 2003 (9, 12).

In Laos, dengue has recently become a recognized public health problem, being the second leading cause of death, with an alarming rising trend in dengue outbreaks every year. In the year 2010, a total of 22,890 dengue cases (including 46 deaths) were reported, and in 2013, the highest number was recorded with total 44,171 dengue cases (including 95 deaths) (10). In 2017, the number of cases and deaths dropped sharply, with 10,102 cases and 14 deaths, making the prevalence of 142 cases per 100,000 inhabitants (13). Major outbreaks, including severe cases and deaths, occur every year, usually during warmer and wetter months, from May to October (14).

Champasack, which is a province in southwestern Laos, near the borders with Thailand and, had the highest number of dengue cases. However, no recent studies have been conducted to identify the key breeding sites at localities with the outbreak of Aedes mosquito larvae in Champasack Province (15). This manuscript is a part of a project on dengue prevention and control conducted in Huaylau village, Pakse city, Champasack province, Lao PDR in 2019. This paper aimed to describe key breeding sites of Aedes mosquitoes in the village and identified factors associated with the absence of the Aedes mosquito larvae and pupae in the village.

METHODS

Study site

The study was undertaken in Huaylau village, Pakse town, Champasack province in Lao PDR in 2019. Champasack province is located in the South of Lao PDR and has its border with Cambodia and Thailand. The province has nine districts and one town (Pakse town). Pakse town locates next to Mekong river and Sedoon river, with 42 villages, an area of 12,508km^2 and the population of 80,515 inhabitants. The majority of the ethnic community in the town is Laos people, some of those are Vietnamese and Chinese people. The climate is tropical monsoon with two distinct seasons, the dry and rainy seasons. The mean annual minimum and maximum temperatures in dry season are 14.6 and 38.3^{0}C respectively, while the mean annual temperature ranges from 23.4 and 32.2^{0}C in rainy season. The precipitation volume of 1,888mm per year (16). The map of the town is represented in Figure 1.

Figure 1. Map of Pakse town, Champasack province

(Source: http://www.ecotourism-laos.org/maps_champasak.htm)

Huaylau is a village of Pakse town, with a favorable condition for transportation by cars or motorbikes. The village has an area of 86.25km^2 and a population of 1,557 inhabitants. Women accounted for 51.4% of the village population. There were 240 households in the village, and the main occupation of the local community was farmers, followed by sale people and government officers. All people in the village were Buddhist (17).

Study population: The study population of this study included one representative (aged 18+) from each of all households in Huaylau village; Aedes larvae and pupae in all water containers and discarded containers of the participated households, and the houses of all households in the study.

Study design and timeline: This was a cross-sectional study, which was undertaken from February to August 2019. Aedes mosquito immature stage survey was conducted in April 2019 in all water and discarded containers indoors and outdoors of the studied households.

Sample size: The sample size for the larvae/pupae survey was all water containers and discarded containers found in all 240 households in Huaylau village. The study respondents included 240 representatives aged 18 years old and older, one from each of the 240 households in the village.

Study procedure: An observational checklist was used to observe hygiene conditions of the households (households were considered to be clean when they were ventilated, illuminated with sunlight during daytime, tidied up with no discarded containers inside or outside of the houses by the time of observation) (18). Mosquito larvae and pupae were collected by entomologists of the Department of Health of Champasack province (DOHC), using pipets or nets depending on the volume of waters in the containers following the entomological survey guidelines issued by Ministry of Health of Laos (19). Then all the collected larvae and pupae were brought to the DOHC's laboratory. At the laboratory, the identification of Aedes larvae and pupae were undertaken. Larvae and pupae were identified morphologically, and larval indices were calculated. Face-to-face interviews were undertaken with the study respondents to obtain information on their knowledge and practices on the prevention of dengue fever. Each correct answer was given one point, and the maximum scores for knowledge and practices were 25 and 10, respectively. The higher score represented better knowledge and practices of the participants on dengue prevention.

Variables

The dependent variable of the study was the absence of Aedes larvae in water containers or discarded containers in households in Huaylau village.

The independent variables of this study included the demographic characteristics of the study participants (gender, age, occupation, educational level, and ethnic group), access to information, education and communication (IEC) campaign about dengue prevention and control, their knowledge and practices on the prevention of dengue, types of houses and hygiene conditions of their houses.

Data analysis

The collected data was uploaded to computer using Epidata version 3.1 software and then transferred to SPSS version 16.0. Descriptive analysis was used to present the general characteristics of the study participants as well as their knowledge and practices on dengue prevention, types of houses and hygiene conditions of their houses and their access to IEC campaign about the prevention and control of dengue. Multivariate logistic regression was applied to identify associations between the independent variables (participants' demographic characteristics, knowledge and practices on dengue prevention, and access to IEC campaign on dengue prevention, their house types and hygiene conditions) and the dependent variables (the absence of Aedes larvae in water containers and discarded containers). Adjusted odd ratio and 95% confident interval were presented. Statistical significance was set at a p-value less than 0.05.

Ethical consideration

Ethical clearance of this study was granted by the Institutional Review Board of the Hanoi University of Public Health on 23rd April 2019 under the Decision number 218/2019/YTCC-HD3.

RESULTS

Of the study respondents, female accounted for 66.2%; 70.8% were 35+ years old. The majority of them were Lao ethnic. 44.6% were farmers, and the larger population had educational level at primary education (30.4%), followed by secondary education (26.3%). 92% of them reported to hear about DF and dengue hemorrhagic fever (DHF).

Key breeding sites of Aedes mosquito in Huaylau village

The key breeding sites of Aedes aegypti is reflected in Table 1. Altogether, 720 containers were observed, of which 52 had Aedes aegypti larvae and pupae. Among the number of containers infested with Ae. aegypti larvae and pupae, discarded containers were the most abundant with 40.6%, followed by flower vases with 22.2% and plastic drums with 21.1%. With regards to Ae. aegypti larvae and pupae infestation in the selected containers, water jars and plastic drums were the most preference for breeding of the mosquito with 34.2% and 30.7%, respectively. On the other hand, buckets (5.2%) and flower vases (2.6%) had lower infestation for Aedes aegypti.

Table 1. The key breeding sites of Aedes aegypti in Huaylau village, 2019

Types of water containers or discarded containers	Number of containers	Number of containers infested with Ae. aegypti larvae	% containers infested with Ae. aegypti larvae	Number of Ae. aegypti larvae found	Prevalence of Ae. aegypti larvae found in containers (%)
Tank ≥ 500 litter	34	0	0	0	0
Tank < 500 litter	192	7	3.6	91	14.7
Water jars	310	15	4.8	212	34.2
Plastic drums	52	11	21.1	190	30.7
Buckets	91	4	4.4	32	5.2
Flower vases	9	2	22.2	16	2.6
Discarded containers	32	13	40.6	78	12.6
Total	**720**	**52**	**7.2**	**619**	**100**

Table 2 represents the key breeding sites of Aedes albopictus in water and discarded containers in Huaylau village. Key breeding site of larvae and pupae of this mosquito was found in discarded containers only.

Table 2. The key breeding sites of Aedes albopictus in Huaylau village, 2019

Types of water containers or discarded containers	Number of containers	Number of containers infested with Ae. albopictus larvae	% containers infested with Ae. albopictus larvae	Number of Ae. albopictus larvae found	Prevalence of Ae. albopictus larvae found in containers (%)
Tank ≥ 500 litter	34	0	0	0	0
Tank < 500 litter	192	0	0	0	0
Water Jars	310	0	0	0	0
Plastic drums	52	0	0	0	0
Buckets	91	0	0	0	0
Flower vases	9	0	0	0	0
Discarded containers	32	4	12.5	35	100
Total	**720**	**4**	**0.05**	**35**	**100**

Totally, the study found 654 larvae and pupae of Aedes mosquito in the 720 surveyed containers in the village. Only larvae and pupae of Aedes aegypti and Aedes albopictus were identified with Aedes aegypti larvae and pupae accounting for 94.6% of sample (Figure 2). Detected breeding sites were categorized into seven groups including Tank ≥ 500 litter, Tank < 500 litter, water jar, plastic drums, buckets, flower vases along with discarded containers.

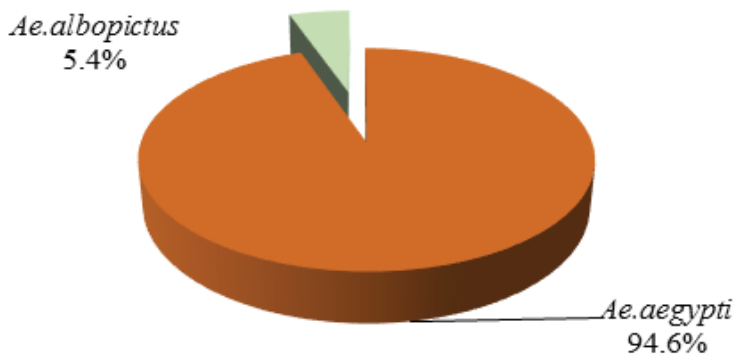

Figure 2. Aedes aegypti and Ae. albopictus larvae and pupae prevalence in Huaylau village, 2019

Factors associated with the absence of Aedes larvae and pupae in water and discarded containers in Huaylau village's households

Factors associated to the Aedes larvae are presented in Table 3. The absence of the Aedes larvae with an odd of 1.612 was witnessed when the score of dengue

prevention practice increased by 1 point (95%CI: 1.218-2.134). In addition, when good cleaning practices were applied, the odds of absent Aedes larvae increased by 4.072 times (95%CI: 1.589-10.434).

Table 3. Factors associated with the absence of Aedes larvae in households in Huaylau village, Pakse city, 2019 (n=240)*

Variables	Adjusted Odd Ratio (aOR)	95%CI	p value
Age	0.998	0.959-1.039	0.929
Gender (female vs male)	1.915	0.769-4.765	0.163
Education (upper secondary level and above vs lower secondary level)	1.069	0.632-1.807	0.804
Occupation (farmers vs other jobs)	0.698	0.374-1.301	0.258
Access to IEC on dengue prevention (yes vs no)	1.437	0.361-5.721	0.607
Knowledge score of the investigated participants on the prevention of dengue	1.039	0.903-1.195	0.595
Practice score of the investigated participants on the prevention of dengue	1.612	1.218-2.134	0.001
Types of houses (1-storey houses and higher vs temporary houses)	1.139	0.530-2.449	0.739
Hygiene condition of households (clean vs dirty)	4.072	1.589-10.434	0.003

** The reference category for the dependent variable is the absence of Aedes pupae in the participants' households.*

DISCUSSION

The study showed that mosquito larvae and pupae of Ae. aegypti and Aedes albopictus were found in water and discarded containers in participated households in Huaylau village. This study's distributions of Ae. aegypti and Ae. albopictus were quite similar to that of previous studies in neighboring countries such as Vietnam (20) and the Philippines (21) or Ethiopia (22), where Aedes aegypti is the dominant vector for DF and DHF(20-22).

Our study revealed that the key breeding sites of Ae. albopictus were probably discarded containers (Table 2), while that of Ae. aegypti included both clean water and discarded containers (Table 1). These results were relevant to that of an entomological study conducted in the Philippines, where the immature stages of Ae. aegypti were found in various containers, including discarded tires and other types of discarded containers(21). Besides, results found by this study were also similar to the results found in a Vietnam study, where many artificial water containers and discarded containers were infested with Ae. aegypti larvae and pupae, while Ae. albopictus could be found only in discarded containers and

in artificial aquarium containers (20). A similar distribution was also observed in an Ethiopian entomological survey for dengue vector control which reported that various artificial water containers and discarded containers were the breeding sites of Ae. aegypti (22). The water jars, water tanks < 500 litter were commonly used for water storage by the local people and were the most important key breeding sites for dengue transmitted mosquitoes in this study. This result was consistent with that of previous studies conducted in Bangladesh (12) and Vietnam (20). From these results, it is implied that the management of water containers and discarded water containers to eliminate the breeding sites of Aedes mosquitoes is important for the prevention of DF and DHF, as suggested by various dengue intervention programs in other countries (23, 24). The study results also suggested that the community level management and control of dengue in this village should focus on the clearance of all discarded containers, as well as the education of local people to cover water containers and/or put fishes or mesocyclops inside water containers (23, 24).

This study revealed that better practices on the prevention of dengue fever at community level and the cleanliness of the house were associated with absence of the Aedes larvae and pupae in the studied households in the village (Table 4). This was consistent with the study conducted in Vietnam in 2017, which concluded that the better implementation of vector control program is the contribution factor to the decline of Aedes larvae and pupae in the studied areas (20). The study results were also relevant to that of Vu Sinh Nam et al. (2012) which reported that after a community based intervention program, the practices of the local community on dengue prevention increased and no larvae was found in water and discarded containers in the study areas, while Aedes larval and pupae population was abundant in water and discarded containers at pre-intervention time (23). The study recommended that appropriate practices on dengue prevention, including keeping the house clean and clear, removing all discarded containers, covering all water containers and regularly changing of water in flower vases should be promoted in the local area to prevent the disease.

Although some valuable results have been found, this study also contained some limitations. Firstly, our study could only investigate the immature stages of Aedes mosquitoes due to some financial, time and human resource limitations. Understanding the distribution of the adult mosquitoes may contribute to the better control and management of Aedes mosquitoes, thus contribute to the decline of DF and DHF cases in communities, as suggested by other studies in the world (24). In addition, this study did not survey the prevalence of DF/DHF cases and deaths in the village at the time and therefore we could not indicate the association between the presence of Aedes larvae and pupae and the prevalence of DF/DHF cases and deaths in the studied population. Future studies should focus on the limited aspects of this study.

CONCLUSIONS AND RECOMMENDATIONS

In Huaylau village, Pakse city, Champasack province, Lao People Democratic Republic, 2019, the key breeding sites of Aedes aegypti were various water containers and discarded containers while all the key breeding sites of Aedes albopictus was discarded containers.

Better practices on dengue prevention and cleaner houses contributed for the increased

chances of Aedes larvae and pupae absence in discarded containers and water containers. Removing discarded containers, covering water containers, regular changing water in flower vases in the households, and keeping the houses clean are appropriate solutions for the prevention of DF and DHF in the village.

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Conflicts of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this manuscript.

Contribution

All the authors contributed equally to the data analysis and writing of this manuscript. Huong LTT, Saenthavisouk V. and Nam VS. developed the proposal and the study protocol. Saenthavisouk V. and Huong LTT analyzed data of the study. Huong LTT and Anh NQ wrote the manuscript. Nam VS edited and finalized the manuscript.

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