Acta Tropica 126 (2013) 177–185

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Relationship between *Aedes aegypti* production and occurrence of *Escherichia coli* in domestic water storage containers in rural and sub-urban villages in Thailand and Laos

Nsa Dada[a,*], Nanthasane Vannavong[a], Razak Seidu[a], Audrey Lenhart[b], Thor Axel Stenström[a,c], Theeraphap Chareonviriyaphap[d], Hans J. Overgaard[a]

[a] Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Drøbakveien 31, 1432 Ås, Norway
[b] Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom
[c] SARChl Institute for Water and Waste Water Treatment (IWWT), Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa
[d] Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form 15 February 2013
Accepted 24 February 2013
Available online 13 March 2013

Keywords:
Aedes aegypti
Domestic water containers
Escherichia coli
Fecal contamination
Dengue

ABSTRACT

In a cross-sectional survey in one rural and one suburban village each in Thailand and Laos the relationship between *Aedes aegypti* production and *Escherichia coli* contamination in household water storage containers was investigated. Entomological and microbiological surveys were conducted in 250 and 239 houses in Thailand and Laos, respectively. Entomological indices across all four villages were high, indicating a high risk for dengue transmission. Significantly more *Ae. aegypti* pupae were produced in containers contaminated with *E. coli* as compared to those that were not, with the odds of *Ae. aegypti* infested containers being contaminated with *E. coli* ranging from two to five. The level of *E. coli* contamination varied across container classes but contamination levels were not significantly associated with the number of pupae produced. We conclude that the observed relationship between *Ae. aegypti* production and presence of *E. coli* in household water storage containers suggests a causal relationship between dengue and diarrheal disease at these sites. How this relationship can be exploited for the combined and cost-effective control of dengue and diarrheal diseases requires further research.

1. Introduction

Globally, over 783 million people, lack access to safe water (UNICEF and WHO, 2012; United Nations, 2012). Poor access to safe water, alongside poor sanitation and hygiene, have been implicated in many infectious diseases (Bartram and Cairncross, 2010; Cairncross and Valdmanis, 2006; Esrey et al., 1991; Pruss et al., 2002) which cause high morbidity and mortality globally (Yang et al., 2012). Some water-related infectious diseases, such as dengue and diarrheal diseases may be causally related. Dengue is an important arboviral disease resulting in an estimated 50 million cases and 30 000 deaths annually, with 2.5 billion people living in risk areas (Farrar et al., 2007; Garelli et al., 2011). It is transmitted mainly by the highly anthropophilic mosquito *Aedes aegypti*, which breeds mostly in domestic water containers in and around human dwellings (Christophers, 1960; Scanlon, 1965), and less frequently by *Aedes albopictus*, which commonly breeds in natural water holding containers like leaf axils (Hawley, 1988). Diarrheal disease, one of the leading causes of child morbidity and mortality, is caused by a wide range of pathogenic organisms including bacteria, viruses and parasites (Guerrant et al., 1990). An estimated 2 billion cases occur each year, killing up to 1.5 million children (WHO, 2009). Diarrheal disease is most often transmitted via the fecal–oral route, with ingestion of faecally contaminated water as one major transmission pathway (Oswald et al., 2007).

Both dengue and diarrheal diseases are public health priorities worldwide. Domestic water storage containers serve as a common source for both diseases, and may also serve as a good target for combined control. Water collection and storage for domestic use is common in areas with water scarcity or in areas where traditional water storage practices exist, as is the case with traditional rainwater harvesting in many parts of Southeast Asia. Rainwater, a suitable alternative or supplement to other water sources, is usually collected and stored in large concrete jars-up to 2000 L capacity – in Thailand (Hewison and Tunyavanich, 1990), or smaller jars or drums, which is common in the investigated areas in Laos. Water from these containers is transferred into smaller containers such

* Corresponding author. Tel.: +47 9682 7969.
E-mail addresses: dada.nsa@umb.no, nsadada@yahoo.com (N. Dada), anandafet@gmail.com (N. Vannavong), razak.seidu@umb.no (R. Seidu), ajl8@cdc.gov (A. Lenhart), thor.axel.stenstrom@sei-international.org (T.A. Stenström), faasthc@ku.ac.th (T. Chareonviriyaphap), hans.overgaard@umb.no (H.J. Overgaard).

0001-706X/$ – see front matter
http://dx.doi.org/10.1016/j.actatropica.2013.02.023

as earthenware jars, pots, and plastic buckets which are easily accessible for everyday use (Pinfold, 1990). In Thailand and Laos, concrete tanks, constructed to hold water from a piped source also exists and are commonly located in the bathrooms. These tanks usually have multiple domestic uses such as laundry, bathing, anal cleansing and toilet cleaning (Kittayapong and Strickman, 1993). In both countries, domestic water containers have been shown to be major *Ae. aegypti* breeding sites (Chareonviriyaphap et al., 2003; Kittayapong and Strickman, 1993; Koenraadt et al., 2008; Tsuda et al., 2002), and these domestic water containers have also been shown to be contaminated with microbes of fecal origin (Pinfold, 1990; Pinfold et al., 1993).

Diverse microbial communities have been found in *Ae. aegypti* breeding habitats (Ponnusamy et al., 2008b). These microbes play important roles in larval nutrition, and their metabolic products have been shown to increase the number of gravid females attracted to containers, as well as the number of eggs layed (Ponnusamy et al., 2008a). The major groups of bacteria found in *Ae. aegypti* breeding containers are Proteobacteria and Bacteroidetes (Ponnusamy et al., 2010, 2008b). Proteobacteria constitute a wide variety of bacteria including diarrhea causing genera like *Escherichia, Salmonella, Vibrio, Helicobacter*, among others. These bacteria are found worldwide in the environment and are also known to inhabit intestinal tracts of vertebrates. On the other hand, only a small group of bacteroidetes have been seen in feces of warm-blooded animals. Although many studies have been conducted on bacterial components of *Ae. aegypti* breeding containers (Ponnusamy et al., 2008b; Zouache et al., 2011) and their effects on *Ae. aegypti* oviposition (Ponnusamy et al., 2010, 2008a), little has been done on the effect of fecal bacteria on *Ae. aegypti* production. We present results of a cross sectional survey carried out in rural and suburban villages in Thailand and Laos to investigate the relationship between *Ae. aegypti* production and *E. coli* contamination (an indicator of fecal contamination and diarrheal disease risk) in domestic water containers. Thailand and Laos are among the top five countries with the highest incidences of dengue and diarrhea in Southeast Asia (WHO, 2004), and were selected to represent a more developed and less developed country in the region. Our specific objectives were to: (i) determine *Ae. aegypti* infestation levels in selected villages, (ii) identify key *Ae. aegypti* containers, (iii) determine levels of *E.coli* contamination in *Ae. aegypti* infested containers and (iv) determine the effect of *E. coli* contamination on *Ae. aegypti* infestation and production. Results were compared within and between countries.

2. Materials and methods

2.1. Study sites and study design

This study was carried out in one rural and one sub-urban village each in Thailand and the Lao People's Democratic Republic (Laos). The selected villages in Thailand were Ban Waileum, rural (16°10′838″ N 102°28′209″ E) and Ban Han, suburban (16°07′801″ N 102°32′159″ E) in Manchakhiri district, Khon Kaen province. In Laos, the selected villages were Ban Okadnavien, rural (15°55′487″ N 105°31′212″ E) and Ban Lakhonesy, suburban (15°53′459″ N 105°33′957″ E) in Lakhonpheng district, Salavan province. (Fig. 1). Both provinces were selected based on high prevalence of dengue and diarrhea reported during the preceding five years, and ease of travel between countries. The districts with the highest dengue and diarrhea prevalence within these provinces were selected. Further criteria for village inclusion were: >70% of households using domestic water storage containers, and no ongoing dengue and/or diarrhea control programs at the time of survey. Housing density and socioeconomic characteristics, in addition to country-specific classification of rural and suburban villages, were used to distinguish between rural and sub-urban villages.

Field work was conducted from February to May, 2011, for approximately two months in each country. Each eligible house was visited once during the two-month period and collections were carried out if the house was occupied at the time of visit and permission was granted to conduct the survey.

2.2. Entomological survey and water sample collection

Two hundred and fifty houses in Thailand (122 in the rural village and 128 in the sub-urban village), and 239 in Laos (115 in the rural village and 124 in the sub-urban village) were surveyed. All houses in both rural villages (Thailand = 124, Laos = 126), and 130 randomly selected houses from each sub-urban village were initially included, but houses that were not occupied at the time of survey or in which consent was refused were excluded. All domestic water holding containers in each house, excluding rubbish, were counted and examined for immature mosquitoes. Containers found positive for larvae, pupae or both were marked positive and information on container shape, use and material, as well as basic demographic information, were recorded.

Water samples from each labeled container were collected into sterile 100 ml polyethylene terephthalate (PET) bottles containing sodium thiosulfate (samples from Thailand) or 100 ml standard Whirl-Pak® Thio-Bags® (samples from Laos). A composite sample was created by collecting small volumes (approximately 10–25 ml) of water across the entire surface of the container to make up 100 ml. All water samples were transported on ice to a field laboratory for analyses. Following collection of water samples and in situ physicochemical tests, all pupae from mosquito-positive containers were collected, counted and preserved in 70% ethanol for identification. If only larvae were encountered, a sample containing ten larvae, or all larvae if less than ten, was taken and also preserved for identification. Physicochemical parameters such as temperature, pH and dissolved oxygen were recorded in situ, while tests for turbidity, ammonia, nitrite and chemical oxygen demand were performed in the laboratory. Effects of these physicochemical parameters and other container characteristics on *Ae. aegypti* and *E. coli* co-infestation will be reported in a subsequent article.

2.3. Mosquito identification and microbiological water analysis

Larvae and pupae were identified using a dissecting microscope and illustrated keys (Bangs and Focks, 2006; Rattanarithikul et al., 2010; Rattanarithikul and Panthusiri, 1994). All *Aedes* pupae were identified to species, while other mosquito pupae were identified to the genus level. For containers that were positive for only larvae, a container was scored positive for *Ae. aegypti* if at least one larvae was identified as *Ae. aegypti*. Any remaining larvae were not further analyzed.

Water samples were analyzed for *E. coli* within 24 h of collection using the Colisure-Quantitray/2000 method (Colisure® WCLS2001, IDEXX laboratories, Inc., Westbrook, USA). Samples were processed according to the manufacturer's instructions (Colisure product insert; IDEXX Laboratories, Inc.) and incubated at 35 ± 0.5 °C. Results were read between 24 and 48 h and recorded as Most Probable Number (MPN)/100 ml. Distilled water was run as negative control.

2.4. Data analyses

Descriptive statistics, including *Ae. aegypti* entomological indices and percentage of containers positive for both *Ae. aegypti* and *E. coli* were computed for each village and country,

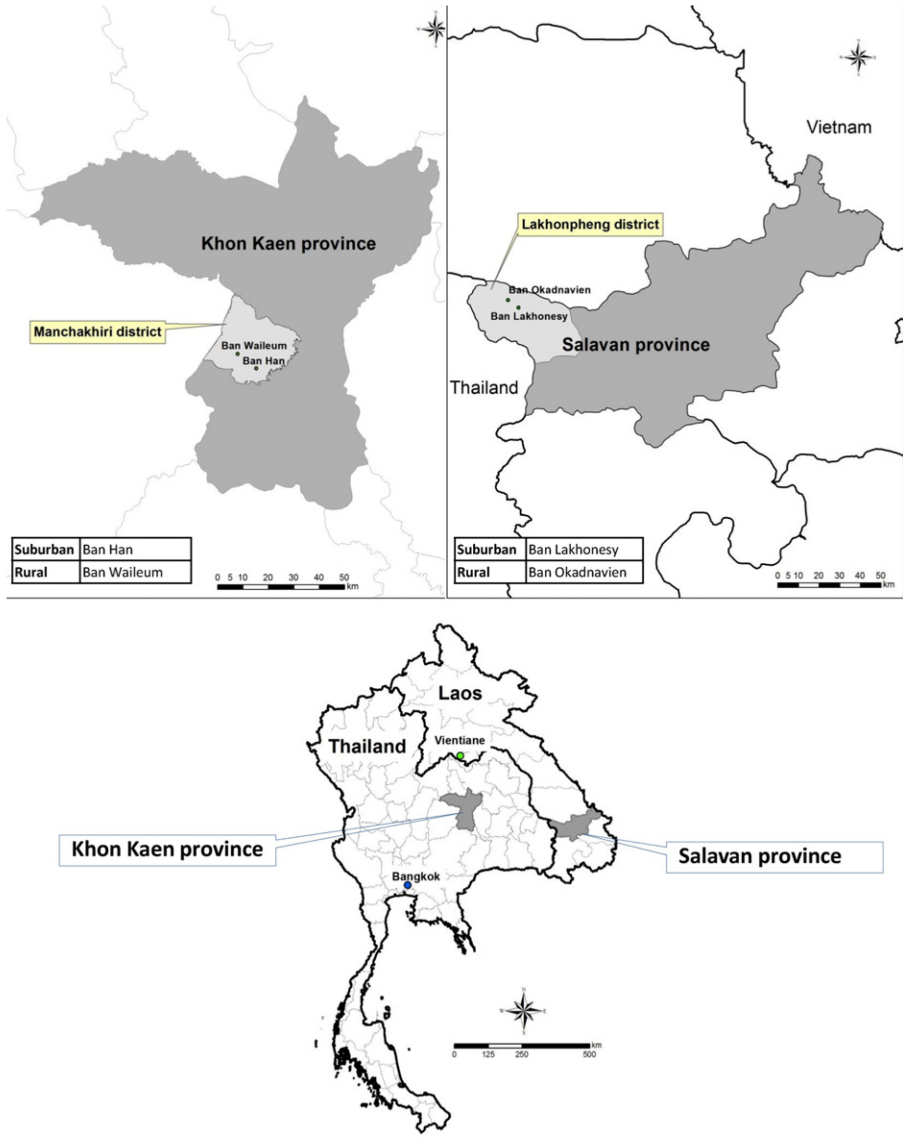

Fig. 1. Locations of study villages in Thailand and Laos.

respectively. Outcomes within and between countries were compared using Pearson's chi square tests.

To identify key *Ae. aegypti* containers and their level of fecal contamination, pupae positive containers were classified according to shape, use and material as adapted from Koenraadt et al. (2007). Resulting combinations of shape, use and material are hereafter referred to as container classes. For each container class, total containers positive for pupae, total number of pupae, container productivity (percentage of total pupae produced by each container class), cumulative container productivity, total containers positive for *E. coli* and mean *E. coli* MPN/100 ml were computed for each village and country, respectively.

Level of *E. coli* contamination in *Ae. aegypti* infested containers was grouped into five categories; <1 MPN/100 ml (very low), 1–10 MPN/100 ml (low), 11–100 MPN/100 ml (moderate), 101–1000 MPN/100 ml (high) and >1000 MPN/100 ml (very high). The very low category i.e. <1 MPN/100 ml, were below detection limit. Number of *Ae. aegypti* pupae positive containers and the average number of pupae collected in each category were computed for each village and country. Chi square tests to compare number of pupae positive containers between levels of *E. coli* contamination, and the Kruskal–Wallis test to compare number of pupae produced between the five levels of *E. coli* contamination were performed at both village and country levels. Mann–Whitney test, using the very low (<1 MPN/100 ml) *E. coli* contamination level as reference, was further performed to determine differences between levels of *E. coli* contamination.

To determine the effect of *E. coli* on *Ae. aegypti* infestation and productivity, the following models were run; (1) binary logistic regression to assess the odds of *Ae. aegypti* infested containers being contaminated with *E. coli* and (2) linear regression to determine the relationship between number of pupae and *E. coli* concentration. In the former model, both dependent (*Ae. aegypti* infestation) and independent (*E. coli* contamination) variables were considered as dichotomous-presence/absence for *Ae. aegypti*, and positive (i.e. >1 MPN/100 ml)/negative (i.e. <1 MPN/100 ml) for *E. coli*. In the latter, both dependent (number of *Ae. aegypti* pupae) and independent (*E. coli* concentration) were considered as continuous, with *E. coli* concentration $\log_{10}$-transformed to reduce skewness. All models were run at both country and village levels. Since there were more than one domestic water containers per house, house ID was included in all models as a random coefficient to account

Table 1
Summary of general findings obtained from the entomological survey and microbiological water analyses conducted in four villages in Thailand and Laos. Rows in bold indicate statistically significant relationships at the 5% level.

Parameter	Thailand			Laos		
Setting	Rural	Suburban	Total	Rural	Suburban	Total
Total no. of houses inspected	122	128	250	115	124	239
No. and (%) of houses with wet containers	121 (99.2)	124 (96.9)	245 (98)	107 (93)	120 (96.8)	227 (95)
No. of residents in inspected houses	435	430	865	636	595	1231
Average no. of residents per house	3.6	3.4	3.5	5.5	4.8	5.2
No. of wet containers encountered	1118	737	1855	491	585	1076
Average wet containers per house	9	6	7	4	5	5
No. of containers with immature *Ae. aegypti*	179	171	350	64	139	203
No. and (%) positive for *E. coli*	129 (72.1)	119 (69.6)	248 (70.9)	40 (62.5)	100 (71.9)	140 (69)
Container index[a]	16	23.2	18.9	13	23.8	18.9
House index[b,x]	**71.3**	**76.6**	**73.6**	**31.3**	**60.5**	**46.4**
Breteau index[c,y]	**146.7**	**133.6**	**140**	**55.7**	**112.1**	**84.9**
No. of pupae positive containers	85	97	182	21	75	96
Total no of pupae	1007	1046	2053	558	911	1469
No. of pupae per person	2.3	2.4	2.4	0.9	1.5	1.2
No. of pupae per house	8.3	8.2	8.2	4.9	7.4	6.2

[a] Percentage of wet containers infested with *Ae. aegypti* larvae and/or pupae
[b] Percentage of houses infested with *Ae. aegypti* larvae and/or pupae
[c] Number of larvae and/or pupae positive containers per 100 houses
[x] Significant difference at country level
[y] Significant difference at both country and village levels

for similarities within houses. Chi square tests were performed to compare number of pupae between *E. coli* positive and negative containers. The level of statistical significance in all analyses was set at 0.05.

All analyses were done using SPSS 20.0 (IBM Corp).

3. Results

Wet containers were found in over 90% (Thailand, $n = 245$; Laos, $n = 227$) of inspected houses. There were more containers in the rural village in Thailand compared to the suburban village, while the reverse was seen in Laos (Table 1). A total of 553 (19%) containers were infested with either *Ae. aegypti* pupae or larvae. These were jars ($n = 295$), tanks ($n = 206$), buckets ($n = 39$), drums ($n = 12$) and one clay pot. Jars were made of either cement or clay (earthen), drums were either plastic or aluminum, and buckets were made of plastic. Tanks were made of either cement (majority of those found in Laos) or tiled, and were located in bathrooms and used for any combination of the following; bathing, brushing teeth, laundry, cleaning toilet or anal cleansing. Jars, drums and buckets had one or a combination of the following uses; drinking, cooking, brushing teeth, washing dishes, laundry, bathing, cleaning toilet and anal cleansing, gardening, and fish storage, or no use (abandoned).

3.1. *Ae. aegypti* infestation in study villages

In both countries, all traditional *Stegomyia* indices were above the accepted thresholds for yellow fever transmission risk, which are still used by dengue control programs around the world to estimate dengue risk (PAHO, 1994). Pupal indices, which are considered better epidemiologic indicators of dengue transmission risk (Focks and Chadee, 1997), were also above dengue transmission thresholds (Focks et al., 2000) in both countries. The house index ($p < 0.05$) and the Breteau index ($p < 0.01$) were significantly higher in Thailand than in Laos (Table 1). In Thailand, the Breteau index was significantly higher in the rural village as compared to the suburban village ($p < 0.01$). Conversely, in Laos, the Breteau index was significantly higher in the suburban village than in the rural village ($p < 0.01$).

3.2. Key *Ae. aegypti* pupae positive containers and corresponding levels of *E. coli* contamination

A total of 40 and 49 container classes were identified in Thailand and Laos, respectively. In Thailand, four container classes, comprising 27% of all pupae positive containers, contained over half of the *Ae. aegypti* pupae collected (Table 2). These four classes included cement tanks and earthen jars with different combinations of uses and one particular cement jar, used as a flower pot, which contained 7% of the total pupae. *E. coli* contamination levels within these four classes ranged from moderate to very high, with the exception of the highly productive flower pot, which was very low (Table 2). Similar container classes contained most of the pupae at the village level, and a similar trend of *E. coli* contamination was found in pupae positive containers in the suburban village. However, the level of *E. coli* contamination of pupae positive containers ranged from moderate to high in the rural village. Irrespective of use or material, tanks contained the most pupae overall (57%), followed by jars (39%) and buckets (4%).

In Laos, six container classes, comprising 31% of all pupae positive containers, contained over half of all *Ae. aegypti* pupae (Table 3). *E. coli* contamination levels within these six classes ranged from moderate to very high (Table 3). At village level, although there were more container classes in the suburban village ($n = 40$) than in the rural village ($n = 15$), most of the pupae were found in similar container classes as described for Thailand. *E. coli* contamination levels ranged from moderate to high in the rural village, and as in Thailand, from moderate to very high in the suburban village. Irrespective of use or material, tanks also contained the most pupae (49%), followed by jars (44%), drums (6%) and buckets (1%).

3.3. Levels of *E. coli* contamination in *Ae. aegypti* pupae positive containers

Across all sites, between 62 and 72% of *Ae. aegypti* infested containers were also positive for *E. coli* (Table 1) and approximately 57% of these containers contained pupae. There was no significant difference in number of pupae positive containers between the five different levels of *E. coli* contamination in both Thailand ($p = 0.96$) and Laos ($p = 0.89$). This was consistent at village level in both countries.

Table 2
Container productivity and level of *E. coli* contamination in *Ae. aegypti* pupae positive containers in Thailand, classified by shape, use and material.

Rank	Shape	Use	Material	*Ae. aegypti*				*E. coli*	
				No. of pupae positive containers	Total pupae	Container productivity[a]	Cumulative productivity	No. of positive containers	Mean ± SD *E. coli* MPN/100 ml
1	Tank	Cleaning toilet and anal cleansing	Cement	28	356	17.3	17	27	490 ± 786
2	Jar	Gardening	Earthen	10	307	15	32	7	176 ± 192
3	Tank	Bathing and laundry	Cement	10	281	13.7	46	9	46 ± 93
4	Jar	Flower pot	Cement	1	153	7.5	54	0	0
5	Jar	Washing dishes	Earthen	18	98	4.8	58	15	97 ± 212
6	Tank	Bathing and brushing teeth	Cement	4	89	4.3	63	4	23 ± 32
7	Tank	Bathing	Cement	6	75	3.7	66	5	323 ± 20
8	Bucket	Cleaning toilet and anal cleansing	Plastic	6	76	3.7	70	5	24 ± 613
⋮									
40	Tank	Laundry	Cement	1	1	0.1	100	1	2
		Total		182	2053			138	

[a] Percentage of total pupae produced by each container class.

However, there were significant differences in the number of pupae between the five different levels of *E. coli* contamination in Thailand ($\chi = 74.2$, $p < 0.01$). This was evident in both rural ($\chi = 37.5$, $p < 0.01$) and suburban ($\chi = 41.3$, $p < 0.01$) villages. In Thailand, most of the pupae were produced in containers with high levels of *E. coli* contamination in the suburban village, but containers with very low levels of contamination produced the most pupae in the rural village (Fig. 2).

In Laos, there were also significant differences in the number of pupae between the five different levels of *E. coli* contamination ($\chi = 58.2$, $p < 0.01$). This significant difference was also evident in both rural ($\chi = 56.4$, $p < 0.01$) and suburban ($\chi = 37$, $p < 0.01$) villages. In Laos, most of the pupae were produced in containers with very low levels of *E. coli* contamination in the rural village, while containers with very high levels of contamination contained most pupae in the suburban village (Fig. 2).

In Thailand, pupae positive containers with very low levels of *E. coli* contamination ($n = 45$) were mostly jars ($n = 29$), followed by tanks ($n = 11$) and plastic buckets ($n = 3$). Only 14 pupae positive containers had very high levels of *E. coli* contamination. These were mostly tanks ($n = 9$), followed by jars ($n = 4$) and a plastic bucket. The majority of the containers with low to high levels of *E. coli* contamination were also tanks, followed by jars and buckets.

As in Thailand, the majority of containers with very low levels of *E. coli* contamination in Laos were jars ($n = 10$), followed by tanks ($n = 2$). Only three tanks and two jars had very high levels of *E. coli* contamination. Low levels of *E. coli* contamination were mostly recorded in jars (17 out of 22), moderate levels of contamination was highest in tanks (15 out of 30), and both jars ($n = 9$) and tanks ($n = 9$) had high levels of *E. coli* contamination ($n = 18$).

In both countries, the majority of jars with very low *E. coli* contamination levels were used for drinking, cooking or both.

3.4. Effect of *E. coli* contamination on *Ae. aegypti* infestation and production

Ae. aegypti infested containers were two to five times more likely to be *E. coli* positive than negative (Fig. 3). This likelihood was significantly higher in Thailand than in Laos, and within villages, significantly higher in each suburban village, respectively (Fig. 3).

In general, linear regression showed no relationship ($r^2 = 0.008$; $p = 0.787$) between number of pupae and *E. coli* concentration. This was neither evident at country nor village level. However, *E. coli* positive containers produced significantly more pupae than *E. coli* negative containers ($p < 0.01$). This was consistent at both country and village levels (Table 4).

4. Discussion

Entomological indices were high throughout the study area. The suggested threshold values for high dengue transmission risk are a Breteau index ≥50 and a house index ≥10 (PAHO, 1994). The Breteau indices in Thailand were almost three times higher than the estimated threshold levels, and up to two times higher in Laos. In Laos and Thailand, house indices were up to six and seven times higher than these estimates, respectively (Table 1). The threshold value of *Ae. aegypti* pupae per person above which dengue transmission occurs has been estimated to be between 0.5 and 1.5 in areas with ambient air temperatures of 28 °C and initial seroprevalences between 0 and 67% (Focks et al., 2000). Thailand and Laos are endemic for dengue (WHO, 2004), and according to each country's meteorological data, average annual temperatures range from 17 °C to 35 °C in Thailand, and 17–34 °C Laos. The overall pupae per person index for Thailand was 2.4 and almost two to five times above this threshold. In Laos the pupae per person index, 1.2, was well

Table 3
Container productivity and level of *E. coli* contamination in *Ae. aegypti* pupae positive containers in Laos, classified by shape, use and material.

Rank	Shape	Use	Material	*Ae. aegypti*				*E. coli*	
				No. of pupa positive containers	Total pupae	Container productivity[a]	Cumulative productivity	No. of positive containers	Mean ± SD *E. coli* MPN/100 ml
1	Tank	Bathing, brushing teeth and laundry	Cement	6	226	15.4	15.4	5	149 ± 99
2	Tank	Bathing, brushing teeth, laundry, cleaning toilet and anal cleansing	Cement	6	139	9.5	25	6	544 ± 946
3	Tank	Bathing, laundry, cleaning toilet and anal cleansing	Cement	4	128	8.7	34	4	138 ± 104
4	Tank	Bathing, brushing teeth, laundry, cleaning toilet and anal cleansing	Tiled cement	6	107	7.3	41	6	216 ± 411
5	Jar	Abandoned/No use	Cement	3	97	6.6	48	2	107 ± 15
6	Jar	Washing dishes	Cement	5	89	6.1	54	5	64 ± 110
7	Drum	Bathing, brushing teeth, dishes and laundry	Steel	3	59	4	58	1	35
8	Jar	Drinking, cooking and brushing teeth	Cement	3	46	3.1	61	3	11 ± 16
9	Jar	Bathing, brushing teeth and laundry	Cement	3	46	3.1	64	3	2 ± 1
10	Jar	Cooking, bathing and washing dishes	Cement	1	45	3.1	67	1	2
...									
49	Jar	Bathing, brushing teeth and washing dishes	Cement	1	1	0.1	100	0	0
		Total		96	1469			81	

[a] Percentage of total pupae produced by each container class.

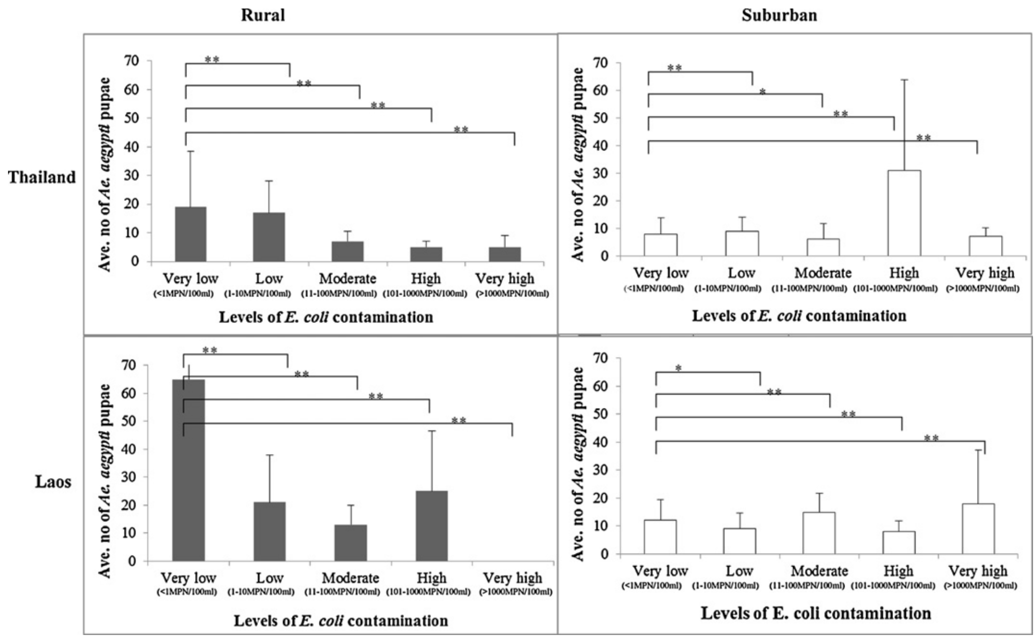

Fig. 2. Differences in average number of *Ae. aegypti* pupae per level of *E. coli* contamination in one rural and one suburban village in Thailand, and Laos using the Mann Whitney test. Error bars represent 95% confidence intervals.
*Significant difference at $p < 0.05$.
**Significant difference at $p < 0.01$.

within the range of the threshold values (Table 1). The high entomological indices can be attributed to the high frequency (>90%) of domestic water storage which has been shown to be a major contributor to the proliferation of dengue vectors (Nguyen et al., 2011). High entomological indices have been reported previously in Thailand (Focks and Alexander, 2006; Luemoh et al., 2003; Thavara et al., 1996) and Laos (Tsuda et al., 2002). Over the years, consistently high entomological indices indicate that *Ae. aegypti* source reduction efforts have not been successful.

Significantly higher Breteau and house indices in Thailand compared to Laos may be explained by a higher proportion of domestic containers in Thailand compared to Laos (Table 1). More containers mean more breeding sites which in turn lead to more mosquitoes. The disparities in Breteau index within countries (higher Breteau index in the rural village in Thailand compared to the suburban village, and the reverse in Laos), could also be attributed to the number of domestic containers in each respective village (Table 1). These *Stegomyia* indices, however, have many shortcomings (Focks and Chadee, 1997) and should be viewed with caution. The pupal index, which may ultimately provide a better indicator of dengue transmission risk (Focks and Chadee, 1997), did not show any statistically significant differences between or within countries.

Irrespective of use, cement tanks contained the most pupae, followed by earthen or cement jars in both countries (Tables 2 and 3) and in all four villages, and these results are consistent with findings from previous studies (Focks and Alexander, 2006; Thavara et al., 2001; Tsuda et al., 2002; Wongkoon et al., 2005). Although level of *E. coli* contamination varied across these container classes, it was not surprising to find that the level of *E. coli* contamination tended to be high in the most pupae productive tanks, because they were located in bathrooms and used for cleaning the toilet and anal cleansing in addition to other uses (among these toothbrushing). Studies have shown that container characteristics such as absence of a cover or lid and low frequency of cleaning are positively correlated with high number of *Ae. aegypti* pupae and increase their odds of infestation (Padmanabha et al., 2010; Spiegel et al., 2007). Unlike most jars, the cement tanks encountered were usually uncovered, not frequently cleaned and located in bathrooms. The highly productive jars in this study were, however, uncovered and also not frequently cleaned. These characteristics make them excellent breeding sites for *Ae. aegypti*, since lack of cover makes them easily accessible for oviposition, and infrequent cleaning leaves sufficient time for immature development.

Across all sites, up to 70% of *Ae. aegypti* infested containers were also positive for *E. coli* (Table 1). This implies that microbes of fecal origin were also present in *Ae. aegypti* domestic breeding containers. Although there was no significant difference between the number of pupae positive containers across the five different levels

Table 4
Difference between total *Ae. aegypti* pupae collected in *E. coli* positive and negative containers in four villages in Thailand and Laos.

Country/village	No. of *Ae. aegypti* pupae in containers				Pearson χ^2	p value
	E. coli positive		*E. coli* negative			
	n	$\bar{X}$ (Min–max)	n	$\bar{X}$ (Min–max)		
Thailand						
Rural	680	46 (1–146)	327	100 (1–153)	813.8	0.000
Sub-urban[a]	795	76 (1–163)	213	33 (1–62)	715.3	0.000
Laos						
Rural	364	42 (2–87)	194	179 (3–186)	538.2	0.000
Sub-urban	763	31 (1–77)	148	24 (1–36)	635.2	0.000

[a] Thirty eight pupae, collected from one container, were excluded from this analysis because the container did not have enough water sample to test for *E. coli*.

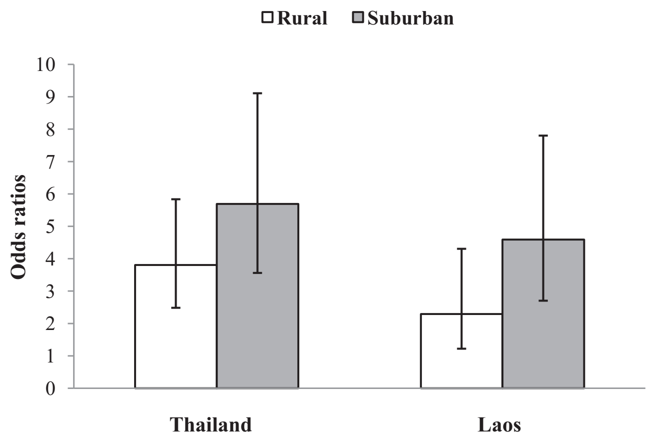

Fig. 3. Odds ratios with 95% confidence intervals of *Ae. aegypti* infested containers being contaminated with *E. coli* in rural and suburban villages in Thailand and Laos.

of *E. coli* contamination, there were significant differences between the number of pupae produced in each level of *E. coli* contamination, and this was evident at both country and village levels. This indicates that pupae were not evenly distributed in pupae positive containers, confirming the suggestion of Focks and Alexander (2006) that pupae are overdispersed. How this overdispersion relates to the levels of *E. coli* contamination remains unclear.

Although there was no correlation between number of pupae and *E. coli* concentration, significantly more pupae were produced in *E. coli* positive containers compared to *E. coli* negative containers. *Aedes aegypti* infested containers were also over two times as likely as non-infested containers to be contaminated with *E. coli*. These results indicate a relationship between pupae production and the presence of *E. coli*. This relationship may be partly accounted for by container use and location, since most of the *E. coli* positive and *Ae. aegypti* positive containers were located in bathrooms, where their use and characteristics increase their chances of fecal contamination and mosquito oviposition. As such, these results suggest a causal relationship between dengue and diarrheal diseases resulting from water storage at these sites. This relationship may be exploited for the combined control of dengue and diarrhea.

5. Conclusion

Ae. aegypti infestation was high across all study villages, suggesting that these villages are at high risk for dengue transmission. Within countries, the rural village in Thailand had higher levels of dengue vector infestation than the suburban village, and vice versa in Laos. Cement tanks were the key *Ae. aegypti* producing containers across all study sites. These tanks were located in bathrooms where their use and other characteristics would be favorable for mosquito oviposition and *E. coli* contamination. The level of *E. coli* contamination varied across container classes but contamination levels were not significantly associated with the number of pupae produced. There was, however, a significant relationship between pupae production and the presence of *E. coli*, suggesting that containers that are contaminated with *E. coli* at any level are more likely to be important dengue vector breeding sites. This suggests a causal relationship between dengue and diarrhea, which may be exploited for the combined control of dengue and diarrhea.

Acknowledgments

This project was funded by the Research Council of Norway (Project no. 191652). The project set up was facilitated by the following; Department of Entomology, Kasetsart University, Thailand; Office of Disease Prevention and Control, Region 6, Thailand; Provincial Health Office, Khon Kaen, Thailand; Ministry of Health, Vientiane, Laos; Provincial Health Office, Champasak, Laos; District Health Office and District Hospital, Manchakhiri; District Health Office and District Hospital, Lakhonpheng; village heads and health volunteers of Ban Waileum, Ban Han, Okadnavien and Lakhonsey.

References

Bangs, M.J., Focks, D.A., 2006. Abridged pupa identification key to the common container-breeding mosquitoes in urban Southeast Asia. J. Am. Mosq. Control Assoc. 22, 565–572.

Bartram, J., Cairncross, S., 2010. Hygiene, sanitation, and water: forgotten foundations of health. PLoS Med. 7, e1000367.

Cairncross, S., Valdmanis, V., 2006. Water supply sanitation, and hygiene promotion. In: Jamison, D.T., Breman, J.G., Measham, A.R., et al. (Eds.), Disease Control Priorities in Developing Countries. , 2nd ed. World Bank, Washington, DC.

Chareonviriyaphap, T., Akratanakul, P., Nettanomsak, S., Huntamai, S., 2003. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Thailand. Southeast Asian J. Trop. Med. Public Health 34 (September (3)), 529–535.

Christophers, S.R., 1960. *Aedes aegypti* (L.) the yellow fever mosquito: its life history. In: Bionomics and Structure. Cambridge University Press, London.

Esrey, S.A., Potash, J.B., Roberts, L., Shiff, C., 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. Bull. World Health Organ. 69, 609–621.

Farrar, J., Focks, D., Gubler, D., Barrera, R., Guzman, M.G., Simmons, C., Kalayanarooj, S., Lum, L., McCall, P.J., Lloyd, L., Horstick, O., Dayal-Drager, R., Nathan, M.B., Kroeger, A., on behalf of the, WHO TDR Dengue scientific working group, 2007. Editorial: towards a global dengue research agenda. Trop. Med. Int. Health 12, 695–699.

Focks, D., Alexander, N., 2006. Multicountry Study of *Aedes Aegypti* Pupal Productivity Survey Methodology: Finidings and Recommendations. WHO/TDR, Geneva, Switzerland.

Focks, D.A., Brenner, R.J., Hayes, J., Daniels, E., 2000. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am. J. Trop. Med. Hyg. 62, 11–18.

Focks, D.A., Chadee, D.D., 1997. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. Am. J. Trop. Med. Hyg. 56, 159–167.

Garelli, F.M., Espinosa, M.O., Weinberg, D., Trinelli, M.A., Gürtler, R.E., 2011. Water use practices limit the effectiveness of a temephos-based *Aedes aegypti* larval control program in northern Argentina. PLoS Negl. Trop. Dis. 5, e991.

Guerrant, R.L., Hughes, J.M., Lima, N.L., Crane, J., 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev. Infect. Dis. 12, S41–S50.

Hawley, W.A., 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 1, 1–39.

Hewison, K., Tunyavanich, N., 1990. Rainwater storage in cement jars in northeastern Thailand. Int. J. Water Resour. Dev. 6, 129–136.

Kittayapong, P., Strickman, D., 1993. Distribution of container-inhabiting *Aedes* larvae (diptera: culicidae) at a dengue focus in Thailand. J. Med. Entomol. 30, 601–606.

Koenraadt, C.J.M., Aldstadt, J., Kijchalao, U., Sithiprasasna, R., Getis, A., Jones, J.W., Scott, T.W., 2008. Spatial and temporal patterns in pupal and adult production of the dengue vector *Aedes aegypti* in Kamphaeng Phet, Thailand. Am. J. Trop. Med. Hyg. 79, 230–238.

Koenraadt, C.J.M., Jones, J.W., Sithiprasasna, R., Scott, T.W., 2007. Standardizing container classification for immature *Aedes aegypti* surveillance in Kamphaeng Phet, Thailand. J. Med. Entomol. 44, 938–944.

Luemoh, A., McNeil, D., Kuning, M., 2003. Water consumption and distribution of dengue larvae in Pattani villages. Songklanagarind Med. J. 21, 209–216.

Nguyen, L.A.P., Clements, A.C.A., Jeffery, J.A.L., Yen, N.T., Nam, V.S., Vaughan, G., Shinkfield, R., Kutcher, S.C., Gatton, M.L., Kay, B.H., Ryan, P.A., 2011. Abundance and prevalence of *Aedes aegypti* immatures and relationships with household water storage in rural areas in southern Viet Nam. Int. Health 3, 115–125.

Oswald, W.E., Lescano, A.G., Bern, C., Calderon, M.M., Cabrera, L., Gilman, R.H., 2007. Fecal contamination of drinking water within peri-urban households, Lima, Peru. Am. J. Trop. Med. Hyg. 77, 699–704.

Padmanabha, H., Soto, E., Mosquera, M., Lord, C., Lounibos, L., 2010. Ecological links between water storage behaviors and *Aedes aegypti* production: implications for dengue vector control in variable climates. EcoHealth 7, 78–90.

PAHO, 1994. Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control. Scientific publication, Washingtong, DC, no. 548.

Pinfold, J.V., 1990. Faecal contamination of water and fingertip-rinses as a method for evaluating the effect of low-cost water supply and sanitation activities on faeco–oral disease transmission. I. A case study in rural north-east Thailand. Epidemiol. Infect. 105, 363–375.

Pinfold, J.V., Horan, N.J., Wirojanagud, W., Mara, D., 1993. The bacteriological quality of rainjar water in rural northeast Thailand. Water Res. 27, 297–302.

Ponnusamy, L., Wesson, D., Arellano, C., Schal, C., Apperson, C., 2010. Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. Microb. Ecol. 59, 158–173.

Ponnusamy, L., Xu, N., Nojima, S., Wesson, D.M., Schal, C., Apperson, C.S., 2008a. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc. Natl. Acad. Sci. 105, 9262–9267.

Ponnusamy, L., Xu, N., Stav, G., Wesson, D., Schal, C., Apperson, C., 2008b. Diversity of bacterial communities in container habitats of mosquitoes. Microb. Ecol. 56, 593–603.

Pruss, A., Kay, D., Fewtrell, L., Bartram, J., 2002. Estimating the burden of disease from water, sanitation, and hygiene at a global level. Environ. Health Perspect. 110, 537–542.

Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E., Richardson, J.H., 2010. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. Southeast Asian J. Trop. Med. Public Health 41 (Suppl. 1), 1–225.

Rattanarithikul, R., Panthusiri, P., 1994. Illustrated keys to the medically important mosquitos of Thailand. Southeast Asian J. Trop. Med. Public Health 25 (Suppl. 1), 1–66.

Scanlon, J., 1965. The distribution of *Ades aegypti* in Thailand. Mosq. News 25, 199–203.

Spiegel, J.M., Bonet, M., Ibarra, A.-M., Pagliccia, N., Ouellette, V., Yassi, A., 2007. Social and environmental determinants of *Aedes aegypti* infestation in central Havana: results of a case – control study nested in an integrated dengue surveillance programme in Cuba. Trop. Med. Int. Health 12, 503–510.

Thavara, U., Tawatsin, A., Chansang, C., Kong-ngamsuk, W., Paosriwong, S., Boon-Long, J., Rongsriyam, Y., Komalamisra, N., 2001. Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. J. Vector Ecol. 26, 172–180.

Thavara, U., Tawatsin, A., Phan-Urai, P., Ngamsuk, W., Chansang, C., Liu, M., Li, Z., 1996. Dengue vector mosquitos at a tourist attraction, Ko Samui, in 1995. Southeast Asian J. Trop. Med. Public Health 27, 160–163.

Tsuda, Y., Kobayashi, J., Nambanya, S., Miyagi, I., Toma, T., Phompida, S., Manivang, K., 2002. An ecological survey of dengue vector mosquitos in central Lao PDR. Southeast Asian J. Trop. Med. Public Health 33, 63–67.

UNICEF, WHO, 2012. Progress on Drinking water and Sanitation: 2012 update.

United Nations, 2012. The Millenium Development Goals Report 2012. New York, pp. 52–53.

WHO, 2004. The World Health Report 2004, Changing History. World Health Organization, Geneva, Switzerland.

WHO, 2009. Diarrheal disease factsheet.

Wongkoon, S., Jaroensutasinee, M., Jaroensutasinee, K., 2005. Larval infestations of *Aedes aegypti* and *Ae. albopictus* in Nakhonsrithammarat, Thailand. Dengue Bull. 29, 169–175.

Yang, K., LeJeune, J., Alsdorf, D., Lu, B., Shum, C.K., Liang, S., 2012. Global distribution of outbreaks of water-associated infectious diseases. PLoS Negl Trop. Dis. 6, e1483.

Zouache, K., Raharimalala, F.N., Raquin, V., Tran-Van, V., Raveloson, L.H.R., Ravelonandro, P., Mavingui, P., 2011. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. FEMS Microb. Ecol. 75, 377–389.