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Philosophiae Doctor (PhD), Thesis 2017:89

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Faculty of Science and Technology

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SUMMARY

The storage of water within the household environment is commonly practiced in many countries in South-east Asia including Laos and Thailand. The practice if not properly managed, can lead to diarrhoea and dengue as a result of faecal contamination and *Aedes (Ae) aegypti* productions of stored water. In Laos and Thailand, diarrhoea and dengue are prevalent. However, the relationship between these diseases and household water storage remains largely obscured and therefore need to be identified for better disease prevention and control. The overall aim of this study was to determine causal factors and health risks associated with faecal contamination and *Ae. aegypti* infestation in household water storage in Laos and Thailand. The specific objectives were to: i) identify risk factors of *Escherichia coli* (*E. coli*) contamination and *Ae. aegypti* pupae infestation in household water storage containers; ii) Assess risk factors and their contribution (Proportion Attributable Fraction (PAF)) to diarrhoeal disease incidence; and iii) Assess the incidence and risk factors of dengue-like illness (DLI), and identify dengue infections.

To address the study objectives, cross-sectional and longitudinal surveys were conducted in one suburban and one rural village each in Laos and Thailand. The purpose of the cross-sectional survey was to gather information on the potential causal factors of *E. coli* contamination in stored drinking water, and *Ae. aegypti* infestation in domestic water containers. The longitudinal had the purposes to collect information on the incidence of DLI and diarrhoeal disease in relation to mentioned risk factors. A total of 478 households were covered in the cross-sectional survey while 2,007 individuals were enrolled in the longitudinal survey. Descriptive analysis and multi-variate regression models were used to describe the characteristics of risk factors, and to find significant relationships between the different risk factors and dengue and diarrhoea in each village.

The cross-sectional survey revealed contamination of *E. coli* in household drinking water as well as high levels of *Ae. aegypti* infestation in the water storage containers in all study villages. Higher *E. coli* concentrations in drinking water were found in Laos than in Thailand, especially in households without toilets (in Laos) and in rural, rather than in suburban villages. The significant risk factors of *E. coli* contamination varied across study villages. In suburban Laos, the significant factors associated with *E. coli* contamination of stored water in

the households were wooden house material, containers (jars and bottles), and households without toilets; whereas in rural Laos the factors were rain-fed water, containers covered with lids and households without toilets. In suburban Thailand significant factors associated with the occurrence of *E. coli* in drinking water were rain-fed containers, jars, buckets and container cleaning frequency; whereas in rural Thailand they were houses made of wood, manually collected rainwater and container cleaning frequency. The study revealed that *E. coli* contamination was less associated with socio-demographic characteristics. Regarding dengue vector production, the number of pupae collected in the study exceeded proposed dengue transmission thresholds of 0.5-1.5 pupae per person. It was shown that most of the household water storage containers in all study villages were without lids, did not contain larvicide temephos and were cleaned less frequently. Household water management and socio-demographic factors were more likely to be associated with *Ae. aegypti* pupae infestation. Factors that were significantly associated with *Ae. aegypti* infestations were tanks, less frequent cleaning of containers, containers without lids, and containers located outdoors or in toilets/bathrooms.

The two-year longitudinal survey on diarrhoeal disease and dengue showed that among 2,007 individuals in the four villages, 97 diarrhoeal cases with no deaths were found comprising 35 and 11 cases in suburban and rural Laos, and 12 and 39 cases for Thai villages, respectively. The study showed that diarrhoeal incidence remains a problem for children under-five years of age, especially in suburban Laos and rural Thailand where the numbers of cases were more frequently reported. The incidences of diarrhoeal disease in under-five year old children in suburban Laos and rural Thailand were 170.5 and 180.0 episodes per 1000 person-years, respectively. The incidence reported in this study was higher than in data from the national surveillance system in both countries. In these settings, the risk factors of diarrhoea were mostly hygiene followed by socio-demographic factors. In suburban Laos, households with children under-five years of age were more likely to report a diarrhoea case (PAF: 58%), followed by households with cooking and feeding utensils left unwashed in the kitchen (PAF: 11%) and households disposing children's stools in the open (PAF: 8%). In rural Thailand, the highest risk attributed to diarrhoea was associated with a delay in cleaning utensils (PAF: 53%).

In addition to diarrhoeal disease, 83 DLI cases were recorded within the same follow up population; these included 69 in suburban Laos, 11 in rural Thailand and 3 in rural Laos (none were found in suburban Thailand). Among these 83 DLI, four (4.8%) were positive for dengue which included two cases each from suburban Laos (DENV serotype 1) and rural Thailand (DENV serotype 2). In suburban Laos, the significant risk factors of DLI were associated with household members within the age cohort of 15-20 years, people's service occupations and the occupation group 'other' (retired and children). In rural Thailand, there were no significant associations between DLI and risk factors in the multivariate model.

The household water storage containers have an impact not only on the microbial deterioration of drinking water, but also on production of immature *Ae. aegypti*. This can potentially lead to further occurrence of both dengue and diarrhoea in these settings. However, the occurrence of DLI was not significantly associated with the observed *Ae. aegypti* pupae per person index, even though this index was generally higher than the proposed dengue transmission threshold of 0.5-1.5 pupae per person.

Based on the results in this thesis, it is recommended that stored drinking water should be treated prior to drinking in order to prevent diarrhoea in households with poor water quality. Provision of toilet facilities in Laos should be made urgently to improve quality of stored drinking water and control against diarrhoea related to improper disposal of the baby stool. Immediate cleaning of utensils after eating or cooking is also important for the reduction of diarrhoea in both Laos and Thailand. Furthermore, to achieve significant reductions in *Ae. aegypti* production and dengue, health education programs should be conducted on the proper use of fitted lids together with weekly cleaning of smaller water containers, as well as on proper use of larvicide temephos strictly in large water-holding containers such as tanks and jars, which were the most infested containers identified in this study. Also, adult mosquito control must also be considered in an integrated vector management strategy. Although compliance is always an issue when it comes to mosquito control, community participation will be key to the success of any selected control measure.

SAMMENDRAG

Oppbevaring av vann i husholdninger er vanlig praksis i mange land i sør-øst Asia inkludert Laos og Thailand. Denne praksis forblir en av de største risiko faktorene for diare og dengue som et resultat av fekal forurensing og *Aedes (Ae) aegypti* produksjon hvis den ikke blir håndtert skikkelig. I Laos og Thailand er både diare og dengue vanlig forekommende. Sammenhengen mellom disse sykdommene og vannlagring i husholdninger er fortsatt uklar og må derfor identifiseres for å bedre prevensjon og kontroll av disse sykdommene.
Hovedmålsettingen til denne PhD oppgave var å bestemme helserisiko i Laos og Thailand forbundet med lagret husholdningsvann som er forurenset fekalt og infisert med *Ae. aegypti* mygg. De spesifikke målsetningene var å 1) identifisere risikofaktorer til mikrobiell forurensning i lagret drikkevann, 2) identifisere risikofaktorer til infestasjon av *Ae. aegypti* pupper i vanntanker i husholdninger 3) vurdere risikofaktorer og deres bidrag til diareinsidens, 4) vurdere insidens og risikofaktorer denguelignende infeksjoner og å identifisere dengueinfeksjoner.

For å løse studiemålene ble det gjennomført tverrsnitts- og longitudinelle undersøkelser i en forstadlandsby (suburban) og en landlig landsby (rural) hver i Laos og Thailand. Formålet med tverrsnittsundersøkelsen var å samle informasjon om de potensielle årsaksforholdene til *E. coli*-forurensning i lagret drikkevann og *Ae. aegypti* angrep i husholdningsvannbeholdere. Den longitudinelle undersøkelsen hadde til formål å samle inn informasjon om forekomsten av denguelignende og diarésykdommer i forhold til nevnte risikofaktorer. Totalt 478 husholdninger i tverrsnittsundersøkelsen og 2,007 personer ble inkludert i den longitudinelle undersøkelsen. Beskrivende analyse og multivariate regresjonsmodeller ble brukt til å beskrive karakteristikker av risikofaktorer, og til å finne viktige forhold mellom de forskjellige risikofaktorene og dengue og diare i hver landsby.

Tverrsnittsundersøkelsen viste høye nivåer av *E. coli* forurensing i drikkevann og høye nivåer av *Ae. aegypti* infestasjon i vannbeholdere i mange husholdninger i alle landsbyene. Høyere *E. coli* konsentrasjonene i drikkevann ble funnet i Laos enn i Thailand, spesielt i husholdninger uten toalett (i Laos) og i landlige landsbyer snarere enn i forstadslandsbyer.

Signifikante risikofaktorer for *E. coli* forurensing varierte mellom landsbyene. I forstads Laos var risiko faktorene assosiert med husmaterial av tre, beholdere (krukker og flasker) og husholdninger uten toalett; mens i landlige Laos var faktorene regn-basert vann, beholdere dekket med lokk og husholdninger uten toalett. I forstads Thailand var de betydningsfulle faktorene assosiert med *E. coli* i drikkevann regnvann i beholdere, krukker, bøtter og rengjøringsfrekvensen av beholdere; mens i landlige Thailand var de husmaterial av tre, manuelt innsamlet regnvann og rengjøringsfrekvensen av beholdere. Studien viste at *E. coli* forurensing var mindre assosiert med sosial-demografisk karakteristikker.

Med tanke på dengue mygg produksjon, så var antallet myggpupper per person høyere enn foreslåtte grenseverdier på 0.5-1.5 myggpupper per person, dermed gir en høy risiko for både diare og dengue infeksjon under disse forholdene. Mesteparten av husholdningenes vannbeholdere i alle landbyene var uten lokk, hadde ingen larvicide temephos, og var rengjort sjeldnere. Håndtering av husholdningsvann og sosial-demografiske faktorer var mer sannsynlig assosiert med *Ae. aegypti* puppe infestasjon. Betydningsfulle faktorer som var assosiert med *Ae. aegypti* infestasjon var vanntanker, beholdere uten lokk og beholdere lokalisert utendørs eller i toalett/bad.

Et toårs studie av diaresykdommer viste at av 2,007 individuelle personer i de fire landsbyene ble det funnet 97 diaretilfeller og ingen dødsfall. Disse inkluderer 35 tilfeller i forstads Laos, 11 i landlige Laos, 12 forstads Thailand, og 39 i landlige Thailand. Studiet viste at diare forekomster er et problem for barn under 5 år gamle, spesielt i forstads Laos og landlige Thailand hvor antall rapporterte tilfeller var høye. Forekomst av diare sykdommer blant barn under 5 år i forstads Laos og landlige Thailand var respektive 170.5 og 180.0 episoder per 1000 persondager. Disse verdiene var høyere enn data fra det nasjonale overvåkelsesystemet i begge landene. Under disse forholdene var risikofaktorene for diare for det meste assosiert med hygiene og deretter sosio-demografiske forhold. I forstads Laos var det for det meste barn under 5 år som var utsatt for diare (PAF: 58%), fulgt av de som hadde uvasket redskap på kjøkkenet (PAF: 11%) og de som kastet avføring fra barn i det åpne (PAF: 8%). I landlige Thailand var den høyeste risikoen med tanke på diare assosiert med utsettelse av vasking av redskap (PAF: 53%).

I tillegg til diare sykdommer, ble det registrert 83 DLI (dengue-lignende infeksjoner) pasienter, hvor 69 var fra forstads Laos, 11 fra landlige Thailand og 3 i landlige Laos (ingen av de ble funnet i forstads Thailand). Av disse 83 DLI pasientene var 4 funnet positive for dengue (4.8%). Det inkluderer 2 tilfeller fra forstads Laos (DENV serotype 1) og landlige Thailand (DENV serotype 2). I forstads Laos, var risikofaktorene for DLI infeksjon assosiert med aldersgruppen mellom 15 og 20 år, sysselsetting (service og 'andre' yrkesgrupper, dvs. pensjonister og barn). I landlige Thailand var det ingen betydningsfulle assosiasjoner mellom DLI og risikofaktorer.

Husholdningsvanntanker påvirker ikke bare mikrobiell forurensing av drikkevann, men også produksjon av *Ae. Aegypti* mygg. Dette kan potensielt føre til ytterligere forekomst av både dengue og diaré i disse områder. Imidlertid var forekomsten av DLI ikke signifikant forbundet med det observerte *Ae. aegypti* puppe per person, selv om denne indeksen var generelt høyere enn den foreslåtte dengueoverføringsgrensen på 0,5-1,5 pupper per person.

I henhold til resultat i denne avhandling, rekommanderes at lagret drikke vann bør behandles før konsumpsjon for å forhindre diare i husholdninger med dårlig drikkevannkvalitet. Anskaffelse av forbedrede toaletter i Laos bør gjøres raskt for å forbedre kvaliteten på lagret drikkevann og kontroll av diaré relatert til feilaktig bortskaffelse av babyavføring. Umiddelbar rengjøring av redskap etter spising eller matlaging er også viktig for å redusere diaretilfeller i både Laos og Thailand. For å oppnå betydningsfull reduksjon av *Ae. aegypti* produksjon og dengue bør helseopplæringsprogrammer utføres på riktig bruk av lokk på vannbeholdere sammen med ukentlig rengjøring av mindre vannbeholdere, samt om riktig bruk av larviciden temephos for bruk i store vannbeholdere som tanker og krukker som var de mest infiserte beholderne i denne studien. Kontroll av voksne mygg må også vurderes i en integrert vektorkontroll strategi. Selv om overholdelse alltid er et problem når det gjelder myggkontroll, vil samfunnsdeltakelse være nøkkelen til suksess til hvilken som helst kontrollmåte.

LIST OF PAPERS

Paper I:

- **Paper I** : N. Vannavong, H. J. Overgaard, T. Chareonviriyaphap, N. Dada, R. Rangsin, A. Sibounhom, T. A. Stenström, R. Seidu (2017). Assessing factors of *E. coli* contamination of household drinking water in suburban and rural Laos and Thailand. *Water Science & Technology: Water Supply* (In production). DOI: 10.2166/ws.2017.133.

- **Paper II** : Vannavong N, Seidu R, Stenström TA, Dada N, Overgaard HJ (2017). Effects of socio-demographic characteristics and household water management on *Aedes aegypti* production in suburban and rural villages in Laos and Thailand. *Parasites & Vectors* 10(1): 170. DOI: 10.1186/s13071-017-2107-7.

- **Paper III** : Nanthasane Vannavong , Hans J Overgaard, Thor Axel Stenström, Razak Seidu. Assessing the risk factors of diarrhoeal disease in suburban and rural villages in Laos and Thailand. (Manuscript).

- **Paper IV** : Nanthasane Vannavong, Razak Seidu, Thor-Axel Stenström, Nsa Dada, Hans Jørgen Overgaard. Dengue-like illness surveillance: a longitudinal survey in suburban and rural communities in Laos and Thailand. *Western Pacific Surveillance and Response Journal* (submitted: 28.9.2017).

LIST OF ABBREVIATIONS

Ae. aegypti: Aedes aegypti

Ae. albopictus: Aedes albopictus

CFU: Colony-Forming Unit

DENV 1-4: Dengue virus 1-4

DIADEN: Diarrhoea and Dengue

DLI: Dengue-like illness

E. coli: *Escherichia coli*

IRR: Incidence Rate Ratio

MPN: Most Probable Number

OR: Odd Ratio

PAF: Population Attributable Fraction

PCA: Principal Components Analysis

RT-PCR: Reverse Transcription Polymerase Chain Reaction

SES: Socio-Economic Status

SRRT: Surveillance and Rapid Response Team

UN: United Nations

WHO: World Health Organization

ZINB: Zero-inflated negative binomial

TABLE OF CONTENTS

PREFACE

This work is part of the project "***Link between Diarrhoea and Dengue: Fecal contamination and dengue mosquito production in household water containers in South-east Asia***" funded by the Research Council of Norway (Project No: 191652). The research was conducted in Lao People's Democratic Republic (Lao PDR) and Thailand in South-east Asia. The work aimed at assessing impacts of household water management practices, socio-demographic characteristics, sanitation and hygiene on faecal contamination (*E. coli*) and *Aedes aegypti* infestation in household water storage containers in one suburban and one rural village in Laos and Thailand, as well as their contribution to the risks of diarrhoeal disease and dengue fever as outcomes. The results from this work may provide important information for the further development of integrated approach to controlling water-borne diseases related to domestic water storage. In addition, the findings can help in addressing not only the increasing threat of dengue but also other arboviral-related diseases, especially in light of the recent spread of Zika outbreaks.

Diarrhoea and Dengue (DIADEN) Midterm Workshop. Rachawadee Resort & Hotel, Khon Kaen, Thailand, 25-27 January, 2012

(A) (B) (C)

(D) (E) (F)

Figure 1. Outdoor household water containers. **A**), Rain-fed container, **B**). Large (2000 liters) and small (200 liters) rain-jars, **C**). Manual drinking water collection from a rain-jar, **D**). Rainwater collection with a hose, **E**). Borehole water collection and storage without lids, **F**). Rainwater without lids

(**A**) (**B**)

(**C**)

Figure 2. Indoor household water containers. **A**), Small rain-jars with lids and scooping cups on top, **B**). Bucket and purchased bottled water, **C**). Jars and tanks in the toilets used for non-drinking water purposes

(**A**)

(**B**) (**C**) (**D**)

Figure 3. **A**). Trainings the project assistants for entomological survey, water and blood sample collections in Laos and Thailand, **B**). Interviewing household head, **C**). Wooden house, **D**). Cement-wooden house

1. INTRODUCTION

1.1. Water-related diseases

Water-related diseases, especially diarrhoea and dengue, remain major contributors to global disease burdens (Hotez *et al.* 2014; Centers for Disease Control and Prevention 2015a). Several risk factors account for diarrhoea and dengue, among which the interlinked factors of household water management, poor sanitation and hygiene practices and socio-demographic characteristics have been identified as major determinants of both diarrhoea and dengue (Reiter *et al.* 2003; Umezaki *et al.* 2007; Brown *et al*. 2008; Genser *et al.* 2008; Koyadun *et al.* 2012; Seidu *et al.* 2013; Cronin *et al.* 2016; Bawankule *et al*. 2017; Guo *et al.* 2017). Globally, 2.3 billion people do not have access to improved sanitation facilities[1], among which 892 million practice open defaecation (WHO 2017a). Although significant progress has been made in access to improved drinking water sources[2] (WHO 2017a), it is estimated that 2.1 billion people still depend on drinking water sources contaminated with faeces, a risk factor for several diseases such as dysentery, cholera, typhoid, hepatitis A, polio etc. (WHO 2017a). More work needs to be done to ensure access to safe drinking water, sanitation and hygiene for all by 2030 as stated in the Sustainable Development Goals (United Nations 2015). Inadequate access to improved water sources in resource-poor countries, as well as problems of intermittent delivery of water supply leads to the storage of water within household environments. Poor household water management practices can result in faecal presence and vector production in stored water that may result in diarrhoea and dengue.

1.2. Household water management, faecal contamination and *Aedes aegypti* infestation

In South-east Asia, storage of water for domestic use is commonly practiced and is ubiquitous in many countries including Laos and Thailand. In Thailand, cement or ceramic jars of all sizes are frequently used as storage containers. Large jars (>2,000 liters) were

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[1] *Improved sanitation facility: A facility that hygienically separates human excreta from human contact (WHO 2017a).*

[2] *Improved drinking water source: A water source that, by nature of its construction or through active intervention, is likely to be protected from outside contamination, particularly from fecal matter (WHO 2017a).*

introduced in the 1980s for storing a sufficient amount of rainwater for long term use. Smaller jars of 200 liters are used in both Laos and Thailand (Pinfold *et al*. 1993; Dada *et al*. 2013; Hiscox *et al*. 2013). Other containers frequently used are cement tanks, drums, plastic buckets etc. Cement tanks are without lids and used to store non-drinking water in the toilets or bathrooms for bathing, laundry and cleaning purposes (See types of water containers in Figures 1 and 2).

A potential link between dengue and diarrhoea risks has been found through a correlation between *Aedes aegypti* pupae infestation and *E. coli* contamination in household water storage containers (Dada *et al*. 2013). Water can become contaminated with diarrhoea-causing bacteria through dirty hands that come into contact with water during collection, transportation and storage (WHO 2002; Clasen & Bastable 2003, Wright *et al*. 2004). A review showed that water from rural areas was more often faecally contaminated than water from the suburban areas (Bain *et al*. 2014a). In addition, water storage containers can also be good breeding sites for *Aedes* mosquito production if not properly managed. This can occur because these containers are not properly covered with lids, do not have any lids at all, are not treated with insecticides, such as temephos (an safe organophosphate insecticide), do not contain larvivorous fish, or are not cleaned on a weekly basis as recommended by WHO (2009).

1.3. Diarrhoeal disease

World Health Organization estimates that diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for nearly 1.7 billion diarrhoeal cases and 525,000 deaths of children every year (WHO 2017b). Most of the diarrhoeal morbidity and mortality occur in lower and middle income countries primarily in Africa and South-east Asia, and are partly due to inadequate sanitation and handwashing practices (WHO 2014a). In South-east Asia, 363,904 diarrhoeal deaths of all ages were reported to be due to inadequate water, sanitation and hygiene (Prüss-Ustün *et al*. 2014). In Laos and Thailand, diarrhoea among children under five ranked as the 5th and 8th leading cause of death, respectively (WHO 2013a, 2013b). Based on reports from the National Center for Laboratory and Epidemiology in Laos (Houatthongkham *et al*. 2016; National Center for Laboratory and Epidemiology, Ministry of Health, Laos [data from 2014-2016 are not available online]), there was an increase in acute watery diarrhoeal incidence from

215/100,000 people in 2009 to 713/100,000 people in 2016. Although in Thailand the incidence rates were higher compared to Laos over the same period, a decreasing incidence was observed from 2,024/100,000 in 2009 to 1,836/100,000 people in 2016 (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, Diarrhoea).

Enteric bacteria, viruses and parasitic pathogens may cause diarrhoeal disease through either faecal-oral transmission route or from person to person contacts as a result of poor hygiene (WHO 2005). Studies conducted in Laos and Thailand found that *Shigella spp*., *E. coli* and *Campylobacter spp*. were frequently detected as pathogenic bacterial etiological agents in addition to rotavirus from diarrhoea patients (Echeverria *et al.* 1994; Yamashiro *et al.* 1998). Although rotavirus accounts for a high proportion of acute gastroenteritis among children in Laos and Thailand (Aloun *et al*. 2009; Chaimongkol *et al*. 2012; Platts-Mills *et al.* 2015; Houatthongkham *et al.* 2016), a rotavirus vaccine is still not included as part of the National Immunization Program in these two countries.

The storage of drinking water within the household environment remains one significant risk factor of diarrhoeal disease (Roberts *et al.* 2001; Dada *et al.* 2013; Günther & Schipper 2013). A significant relationship between diarrhoeal disease and the presence of *E. coli* in household drinking water has been reported in several studies. Jensen *et al.* (2004) and Levy *et al*. (2012) found a significant association between diarrhoeal disease and the presence of *E. coli* in household drinking water. Diarrhoeal disease was associated with *E. coli* levels of ≥ 10 CFU/100 mL and ≥ 11 CFU/100 mL in drinking water respectively in Zimbabwe (Gundry *et al*. 2009) and Cambodia (Brown *et al*. 2008). In addition to faecally contaminated water, diarrhoeal disease can also be caused by the consumption of faecally contaminated food such as uncooked foods of animal origin, fruits and vegetables; rotten food and toxic chemicals (Seidu *et al*. 2013; WHO 2015).

Socio-economic status (SES), sanitation and hygiene are also important risk factors of diarrhoeal disease. For instance, poverty has been found to be associated with unimproved water and sanitation (Blakely *et al*. 2005). A case-control study conducted in a semi-urban area in Thailand showed that those who stayed in the rental houses and had a low family income were significantly associated with shigellosis (Chompook *et al.* 2006). A systematic review found that 42%-47% of diarrhoeal risk in the communities can be reduced through washing hands with soap (Curtis & Cairncross 2003). The frequency of diarrhoea among Mozambican refugee in Malawi was found to be significantly associated with lack of soap (Peterson *et al*. 1998). Another important factor associated with increased diarrhoeal incidence was the presence of animals at homes, where houses that have a high number of sheep were found to be associated with diarrhoea in children under-five in western Kenya (Conan *et al.* 2017). It was evident that households without toilets and improper disposal of stool significantly increased the odds of diarrhoeal episodes in children under-five (Mihrete *et al.* 2014).

1.4. Dengue

Dengue is a mosquito-borne viral infection caused by four distinct serotypes; DENV-1, DENV-2, DENV-3, DENV-4, and is prevalent in tropical and subtropical regions. The global distribution of dengue has shown a dramatic increase during the past 50 years and around half of the world's population is at risk (WHO 2017c). Dengue is prone to spread into new dengue-free areas (Gubler 2011). Recently, a dengue vaccine was developed and several national regulatory authorities, including Thailand (but not Laos), have approved it for public use (Centers for Disease Control and Prevention 2015b; Sanofipasteur 2016). WHO estimated that 50-100 million people are infected by DENV each year in up to more than 100 countries (WHO 2014b). In South-east Asia, one of the largest outbreaks of dengue occurred in 2010, where 22,929 cases and 46 deaths were recorded in Laos (Arima *et al*. 2015) and 116,947 cases and 139 deaths in Thailand (Limkittikul *et al.* 2014). In Thailand, dengue outbreaks also occurred in 2013 and 2015, with 154,444 cases and 136 deaths in 2013, and 144,952 cases and 148 deaths in 2015 (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, Dengue haemorrhagic fever). In Laos, another dengue outbreak occurred in 2013 resulting in 44,171 cases and 95 deaths. There was no outbreak in 2015 in Laos in parallel with the one that occurred in Thailand (WHO 2017d). In the southern part of Laos, dengue outbreaks commonly occur including in Saravan province (Khampapongpane *et al*. 2014). In Thailand, Khon Kaen province (north-east Thailand) is also one of the dengue affected areas; where an entomological survey conducted in 966 rural and urban households (5821 containers) in this province showed high values of Breteau and Container Indices (Phuanukoonnon *et al.* 2005). Based on numbers of dengue cases reported to WHO during 2004-2010, Laos and Thailand were grouped as the 30 most highly endemic countries globally (WHO 2012).

Household water storage containers are recognized as the preferred breeding sites for *Ae. aegypti* in tropical and subtropical regions (Tonn *et al*. 1969; Swaddiwudhipong *et al.* 1992; Tsuda *et al*. 2002; Chareonviriyaphap *et al*. 2003). In Laos and Thailand, water holding containers, such as jars and cement tanks are frequently infested with *Ae. aegypti* (Tonn *et al*. 1969; Kittayapong *et al*. 1993; Tsuda *et al*. 2002; Phuanukoonnon *et al.* 2005; Hiscox *et al*. 2013), as well as elsewhere in South-east Asia (Seng *et al.* 2009; Tsuzuki *et al*. 2009) and in Latin America (Quintero *et al*. 2014). Other domestic storage containers e.g. drums and buckets have also been recognized as major breeding sites of *Aedes* mosquitos in South-east Asia (Chan *et al.* 1971; Tsuda *et al.* 2002; Seng *et al*. 2009; Aldstadt *et al*. 2011). The reasons that these containers are being *Aedes*-infested may be because lids are lacking and that containers are not cleaned often enough. Containers without lids or partly covered produced more *Ae. aegypti* than those with lids (Koenraadt *et al*. 2006; Tsuzuki *et al*. 2009; Hiscox *et al*. 2013; Quintero *et al*. 2014) and those with less frequent cleaning were more likely to be colonized by the dengue vector (Phuanukoonnon *et al.* 2005; Maciel-de-Freitas *et al*. 2007; Arunachalam *et al*. 2010; Tsunoda *et al*. 2014; Ferdousi *et al*. 2015).

In addition, socio-demographic and socioeconomic factors are known to indirectly affect dengue vector production and transmission. For instance, the risk of dengue in Thailand was associated with people with at least secondary education level and with households of more than four members (Koyadun *et al*. 2012). Others found that dengue fever has a strong positive association with population density (Seng *et al.* 2005; Khormi & Kumar 2011). In the border areas of Mexico and Texas, USA dengue seropositivity (immunoglobulin M, immunoglobulin G) was significantly associated with the absence of air-conditioning in households (Reiter *et al.* 2003).

2. RESEARCH AIM, OBJECTIVES, CONCEPTUAL FRAMEWORK AND RATIONALE

2.1. Aim and objectives

The overall aim of this study was to determine causal factors and health risks associated with faecal contamination and *Ae. aegypti* infestation in household water storage in Laos and Thailand. The specific objectives were to:

1. Identify the risk factors of microbial contamination of stored drinking water (**Paper I**).
2. Identify the risk factors of *Ae. aegypti* pupae infestation in domestic water containers (**Paper II**).
3. Assess risk factors and their contribution to diarrhoeal disease incidence (**Paper III**).
4. Assess the incidence and risk factors of dengue-like illness (DLI), and identify dengue infections (**Paper IV**).

2.2. Conceptual framework

The conceptual framework for this thesis is shown in Figure 4. The framework was developed in line with the overall aim and objectives of the study. The framework establishes the linkages between the three major blocks of risk factors accounted for in this study (i.e. socio-demographic characteristics (block A), household water management (block B) and sanitation and hygiene (block C)) and their relationships with the key findings made in the study (**Papers I-IV**). All the three major blocks of risk factors were accounted for in assessing the risk factors of *E. coli* contamination in household drinking water **(Paper I)** and diarrhoeal disease incidence **(Paper III)**, while blocks A and B were accounted for in assessing *Ae. aegypti* pupae infestation of stored water containers **(Paper II)**. From the results of **Paper II**, *Ae. aegypti* pupal indices (pupae per house and pupae per person) together with block A were used for further risk assessment on the occurrence of DLI and dengue infection **(Paper IV)**.

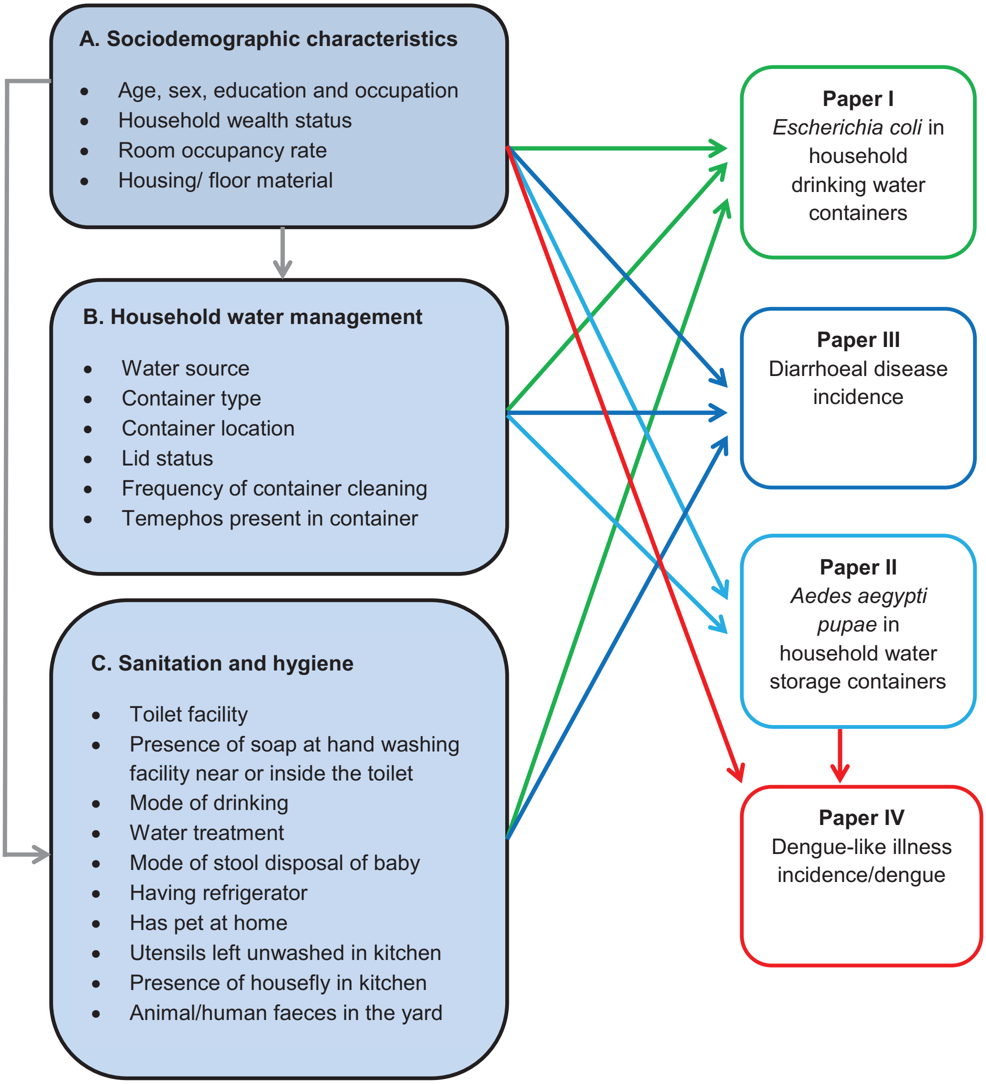

Figure 4. Study framework of the thesis. The components A, B and C in the boxes on the left are risk factors. Other boxes are outcomes or paper lists, where the specific colored lines connected to boxes indicate risk factors assessed in each paper (The green lines represent risk factors assessed for paper I, the light blue lines are for paper II, the dark blue lines are for paper III and the red lines are for paper IV)

2.3. Rationale

The overall rationale of the thesis is to understand how water storage within the household environment could lead to faecal contamination and *Ae. aegypti* infestation, and have further impact on dengue and diarrhoeal diseases. The burden of diseases for both diarrhoea and dengue is high in Thailand and even higher in Laos (WHO 2014c). An enhanced understanding of the impacts of improper water handling could provide a basis for developing integrated control measures against both diseases. Furthermore, longitudinal surveillance of diarrhoea and dengue is rarely conducted in these countries. An investigation is therefore needed to capture the real incidences of both diseases burden by comparing with the secondary data from the national surveillance system.

Several factors affect the quality of stored drinking water in the household environment. These factors include water contamination at the source (Taneja *et al*. 2011; Too *et al*. 2016); during water collection and storage (Jensen *et al.* 2002; Clasen & Andrew 2003) as well as hygiene and handling practices (Gundry *et al.* 2006; Eshcol *et al.* 2009; Rufener *et al*. 2010). There are no studies in South-east Asia, particularly in Laos and Thailand, on the main drivers of microbial contamination of stored drinking water in the household environment. For the development of cost-effective interventions, it is critical that factors contributing to the microbial contamination of drinking water in the household environment are identified. This study accounts for a wide range of factors that can contribute to *E. coli* contamination of stored drinking water in the household environment including socio-demographic, household water management, drinking water, and sanitation and hygiene factors (**Paper I**).

Diarrhoea is a leading cause of malnutrition in children less than five years old. For preventing and ensuring maximum health benefits of these high-risk groups, identifying the risk factors of diarrhoeal disease incidence is important. Diarrhoeal disease has been included in the National Notifiable Diseases of the Ministry of Public Health in Laos and Thailand since 1995 and 1970, respectively. Since then diarrhoeal incidence has been reported as part of the national surveillance data. Diarrhoeal incidence is, however, less well reflected in recorded data from communities among the general population (**Paper III**). The findings from this study could improve the understanding of the effect of household water management practice, socio-demographic characteristics, sanitation and hygiene on diarrhoeal disease in these settings, and for streamlining and developing better control measures of diarrhoea.

Dengue is a mosquito-borne viral infection with no specific treatment. A dengue vaccine, Dengvaxia®, is available, but does not confer full protection to all virus serotypes (WHO 2016). Thailand, but not Laos, has approved this vaccine for public use. Early detection and access to proper medical care has been instrumental in lowering dengue fatality rates from more than 20% to less than 1% (WHO 2017c). The prevention and control of dengue is therefore a priority and normally depends on the effectiveness of dengue vector control applied in a particular area. In Laos and Thailand, mass distribution of the larvicide temephos is routinely applied in combination with other control measures such as larvivorous fish and weekly cleaning and covering of water containers. Although a review showed that temephos was effective against *Ae. aegypti* production (George *et al.* 2015), there is evidence of its inconsistent and improper use as previously observed in Thailand (Phuanukoonnon *et al.* 2006) and this may lead to ineffective control. To date, several studies have been conducted to assess risk factors of dengue vector production associated with household water management and socio-demographic characteristics, but this has rarely been conducted in these settings, particularly in Laos (**Paper II**). The results from this study will provide important information for *Ae. aegypti* control programs to address the increasing threat of arboviral diseases, especially in light of the recent spread of Zika outbreaks. The findings of this study will be used as the baseline information for the further work on the surveillance of dengue or DLI infections (**Paper IV**).

Dengue-like illness (DLI) has been used to describe any dengue case where the patients had similar clinical manifestations of dengue infection. For example, DLI was recorded in some Africa regions where there was limited access in laboratory confirmation for dengue and chikungunya (WHO 2009). In tropics and subtropics of dengue endemic areas, there are many infectious diseases primarily present with similar clinical manifestations such as typhoid fever, leptospirosis, typhus fever, malaria, chikungunya etc., these DLI infections can lead to a confusion with dengue surveillance, prevention and control (Gulati and Maheshwari 2012). In 2014, the Council of State and Territorial Epidemiologists (CSTE) recommended that DLI should be added into the list of nationally notifiable diseases (Centers for Disease Control and Prevention 2015b). The active surveillance of DLI infections and DENV serotypes circulated in non-dengue outbreak situation is rarely reported, particularly in dengue endemic countries in Laos and Thailand, where storage of water at household environment is very common and potentially provides a lot of breeding sites for *Ae. aegypti* infestation. This study assessed the risk factors of DLI infection associated with *Ae. aegypti* pupal indices in the households (e.g. pupae per house and pupae per person) as well as socio-demographic characteristics (**Paper IV**). The study results can provide useful information to develop more effective strategies on dengue prevention and control.

3. MATERIALS AND METHODS

3.1. Study sites and study design

Using existing health data and in consultation with each country's public health departments, one suburban and one rural village each in Laos and Thailand were selected (Figure 5). The selected villages in Laos were suburban Ban Lakhonesy (15°53'29.18"N, 105°33'56.59"E) and rural Ban Okadnavien (15°55'22.37"N, 105°31'35.0"E) in Salavan province, southern Laos (these two villages were approximately 6 kilometers apart). In Thailand, the villages selected were suburban Ban Han (16°07'50.71"N, 102°32'5.81"E) and rural Ban Waileum (16°10'48.95"N, 102°28'15.61"E), Khon Kaen province, northeastern Thailand . These two villages each in Laos and Thailand were approximately six and nine kilometers apart, respectively. The study villages were selected on the criteria that more than 70% of the village households used domestic water storage containers and there was no ongoing dengue and/or diarrhoea control programs at the time of study. The selected villages in Thailand and Laos had a total of 411 households (272 suburban and 139 rural) and 345 households (215 suburban and 130 rural), respectively. A systematic random sampling method was used to select a sample of households in each village. The total numbers of houses in a village was divided by 130 which was the target of the sample size based on the total numbers of households in rural Laos. A random house was selected as a starting point. The additional houses were thereafter selected based on a fixed interval derived from the dividend above.

A cross sectional survey (**Papers I and II**) was conducted from the end of February to the beginning of June 2011, in each of the selected villages in Thailand (Feb-April) and Laos (May-June). The cross-sectional survey involved 248 households in Thailand (127 suburban and 121 rural) and 239 households in Laos (124 suburban and 115 rural). Some households were not included as initially selected (130 households) because the householders were either not present at home during visits for data collection or refused to sign the consent form.

A longitudinal survey on diarrhoeal disease in 2011 to 2013 (**Papers III**) and DLI infection (**Papers IV**) was conducted right after completing the cross sectional survey. The start investigation periods were slightly different depending on the sites (suburban Laos: May 2011-March 2013, rural Laos: June 2011-April 2013, suburban Thailand: April 2011-

February 2013, and rural Thailand: March 2011-January 2013). During the course of the survey, initial registration was made from a total of 2,035 people in the selected households (suburban and rural Laos: 586 and 613 people, and in Thailand: 397 and 439 people). Of these, 78 were lost during the follow up and 50 were newly enrolled. The final number at the end of the two-year follow up period was 2,007 people (Figure 6).

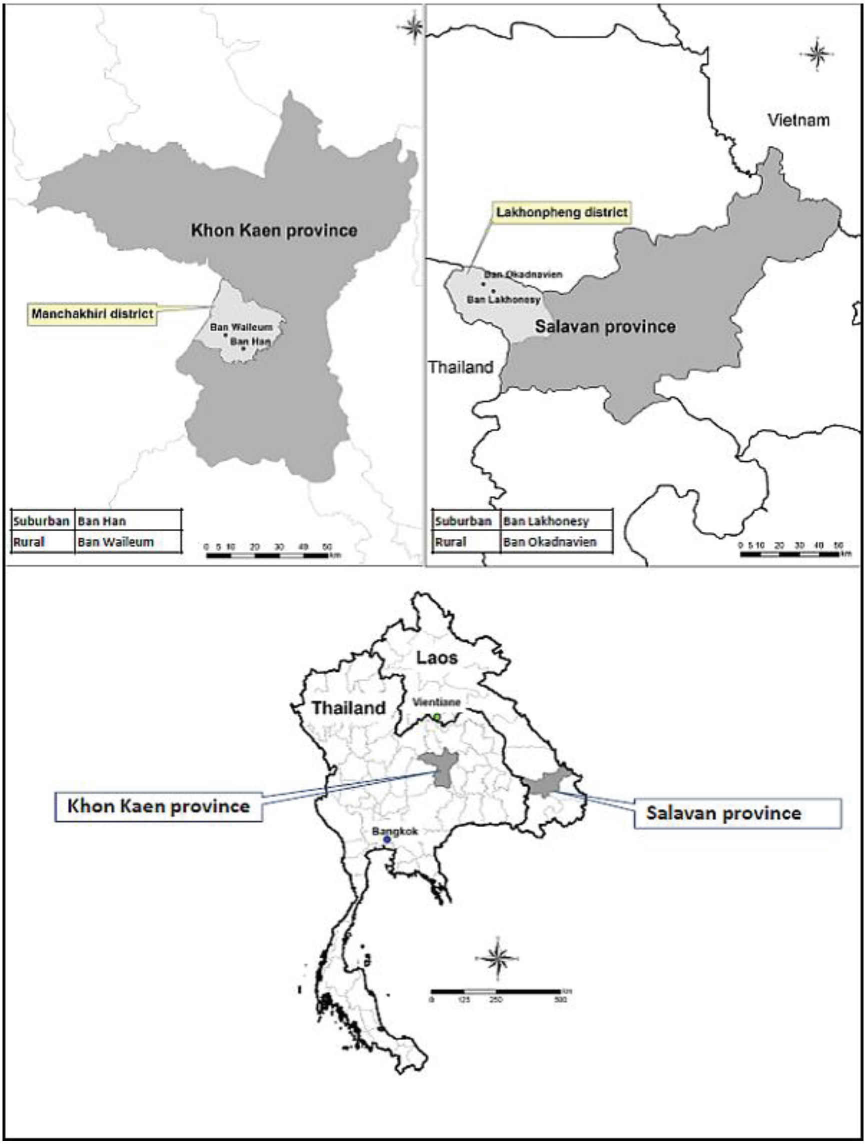

Figure 5. Study villages in Laos and Thailand

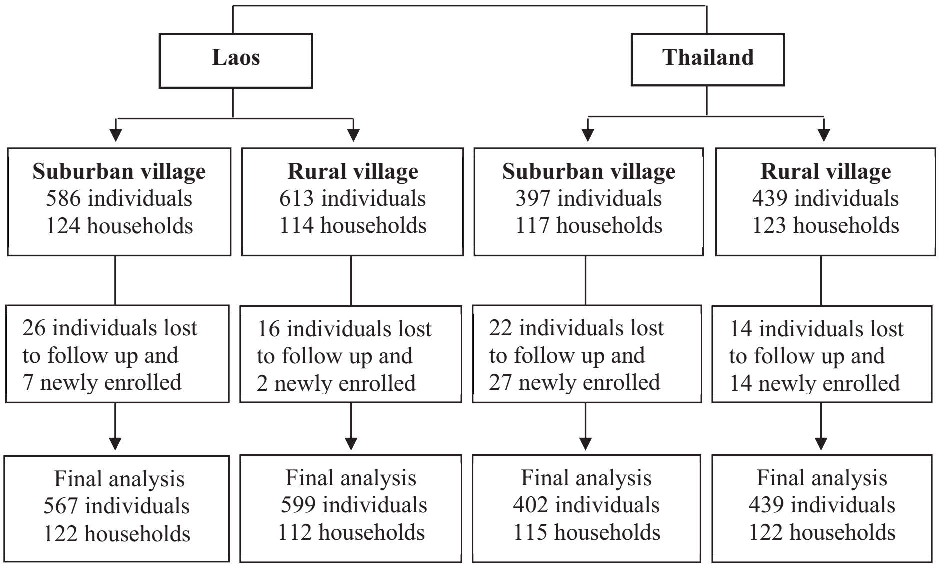

Figure 6. Number of participants and households during the two-year follow up period in four villages (**Papers III and IV**)

3.2. Data collection

After selection of households, the cross-sectional and longitudinal surveys were conducted together with trained field staff, i.e. local village health volunteers (Figure 3).

3.2.1. Socio-demographic characteristics of the households

Semi-structured questionnaires were prepared (in Laos and Thai languages) and used to interview the heads of each selected household (respondents) in the villages (**Paper I-IV**). Personal information of each respondent as well as of all other household members, such as age, sex, education level and occupation, were obtained. Information related to households' ownership of durable assets (mobile phone, cell phone, TV, radio, refrigerator, car, motorcycle, bicycle), habitable room occupancy and access to water was also collected. In addition to the interviews, observations were made of housing material and recorded.

3.2.2. Sanitation and hygienic survey

Information on sanitation facilities and hygiene were collected through interviews with semi-structured questionnaires and observations (**Papers I and III**). Information collected included the types, access and use of toilet facilities, presence of soap at handwashing facilities near or inside the toilets, handwashing after the toilet, presence of any pets/animals at home, presence of animal/human faeces in the yard, mode of stool disposal of the babies or children who don't use toilets, utensils left unwashed, cleaning utensil after eating/cooking, and presence of houseflies in kitchen, and mode of collecting water to drink.

3.2.3. Household water management survey

As part of the household water management survey, all water storage containers were classified according to type, presence or absence of a lid, frequency of refill, and location. The sources of the household water were characterized as rain-fed (rainwater that is collected directly from the rooftop, through roof connected tubes or from metal roofing sheets), manually collected rainwater (rainwater collected manually from larger containers), piped water into the household, or borehole water (i.e. boreholes or protected drilled wells owned by households and located in the housing areas). Containers were defined as being indoors if located under the main roof of the house or outdoors if located outside the house or under the eaves of the house. Containers in bathrooms/toilets were classified as a separate group (i.e. neither indoors nor outdoors).

3.2.3. Drinking water quality survey

Drinking water samples were collected from a total of 139 and 145 water containers in suburban and rural Laos (**Paper I**). In Thailand, 178 and 268 drinking water samples were collected from the suburban and rural villages respectively. Samples were collected using sterile 100 mL Whirl-Pak bags, put on ice and transported to a field laboratory where they were analyzed for *E. coli* within 24 hours after sampling. *E. coli* analysis was done using 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˚C ±0.5˚C. Results were read after between 24-48 hours and recorded as Most Probable Number (MPN)/100 mL. Distilled water was run as negative control.

3.2.4. Entomological survey

All household containers used for water storage were examined for mosquito pupae and larvae (**Papers II and IV**). If present, pupae were collected, counted, and brought back to the field station for identification using a dissecting microscope and illustrated keys as previously described (Dada *et al*. 2013). All *Aedes* pupae were identified to *Ae. aegypti* and *Ae. albopictus.* Only thirteen pupae from the Lao study villages (5 suburban and 8 rural) were identified as *Ae. albopictus.* Therefore, this species was excluded in the analysis. For containers that were positive for only larvae, a container was scored positive for *Ae. aegypti/albopictus* if at least one larvae was identified as *Ae. aegypti/albopictus*. Any remaining larvae were not further analyzed.

3.2.5. Longitudinal survey on follow up of diarrhoeal disease and dengue-like illness (DLI)

Door-to-door visit was conducted weekly by trained village health volunteers in each village (5-7 persons per village) during approximately two years to record diarrhoeal disease (**Paper III**) and DLI infection (**Paper IV**) which occurred among the follow-up household members in the villages of Laos and Thailand. In the survey, diarrhoea was defined as the passage of three or more loose or liquid stools within 24 hours (WHO 2005). A new episode of diarrhoea was considered when it re-occurs at least three days after the first diarrhoea episode had stopped (Baqui *et al.* 1991). In addition, the district-level secondary data of diarrhoeal disease and dengue of the study areas were obtained from the national surveillance system in order to compare with this study. Dengue was defined as the presence of acute febrile illness with two or more of the non-specific symptoms such as headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, and leukopenia pending a serology or isolation of virus (WHO 1997).

If the DLI case was present during each visit the general information of a patient was collected, through a questionnaire form, directly from the case or from mother/caretaker if one was less than 15 years old. Furthermore, a total of 100 μL of blood samples from fingertips of DLI patients were blotted onto two pieces of filter papers by following the manufacturer's instructions (Blood Sampling Paper, NOBUTO, Toyo Roshi Kaisha Ltd., Japan, Type I) (Figure 7). After the blood samples were absorbed, the papers were dried at room temperature for 1-2 hours. After drying, the papers were tightly sealed in sterile Whirl-Pak Bags and kept in a minus 20° C freezer. All the samples were periodically brought to Thailand to identify if DENV was present and further the serotypes of DENV using the Real-Time PCR as described by Prado *et al* (2005). In addition, the stool samples of diarrhoeal cases were also collected during the course of their illness, in a sterile plastic box by using a guideline provided to patients together with the box.

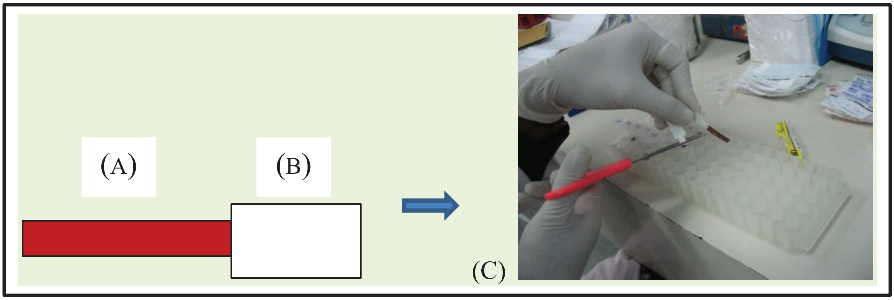

Figure 7. Filter papers employed in the study. Blood absorbing area (A), blood diffusion area (B), and detection of DENV serotypes with RT-PCR analysis (C)

3.3. Data analyses

Descriptive analysis was undertaken to examine the statistical distribution (frequency, percentage, central tendency and rate) of factors related to socio-demographic characteristics, household water management, drinking water, sanitation and hygiene (**Paper I-IV**, but only socio-demographic characteristics and household water management were assessed and used in **Paper II and paper III** analyses). Further analysis was undertaken to derive additional risk factors such as room occupancy rate and wealth status of the households. Estimation of the room occupancy rate was based on United Nation’s definition (United Nations Statistics Division 2016). Households were ranked as rich, intermediate and poor ones using Principal Components Analysis (PCA) and based on group weighted mean scores (Vyas & Kumaranayake 2006). The variables used in the wealth status ranking are presented in Table 1. For faecal contamination in household drinking water, the mean, minimum and maximum of *E. coli* (MPN/100 mL) concentrations related to all study factors were accounted for

(**Paper I**). As for dengue vector production, the proportion of containers positive for *Ae. aegypti* pupae, total number of pupae infested in the household and *Ae. aegypti* pupae per container were calculated (**Paper II**).

Table 1. Variables used in the wealth status ranking **(Papers I and II)**

Variables	Options/Values
House material	Cement/ wooden/ cement-wood
House floor material	Cement/ wooden/ cement-wood
Room occupancy rate	> 2.5 persons per habitable room/ ≤ 2.5 persons per habitable room
Ownership of durable assets	Mobile phone/ cell phone/ TV/ radio/ refrigerator/ car/ motorcycle/ bicycle
Affordability of bottled water	Can afford/ cannot afford
Ownership of toilet facility	Yes/ No
Ownership of flush toilet	Yes/ No
Ownership of pour flush toilet	Yes/ No

A relationship analysis with univariate analysis was undertaken to assess the independent effect of risk factors on the occurrence of *E. coli* in the household drinking water (**Paper I**) and *Ae. aegypti* pupae infestation in the household water storage containers (**Paper II**). For this analysis, the concentration of *E. coli* and *Ae. aegypti* pupae were each used as dependent variable in a zero-inflated negative binomial regression (ZINB). Regardless of significance, all factors included in the univariate analysis were entered into a ZINB multivariate model to assess the effect of multiple factors on the occurrence of *E. coli* and *Ae. aegypti* pupae. From the multivariate ZINB analysis, incidence rate ratios (IRR) and their 95% confidence intervals were obtained. The IRRs of factors significantly affecting ($p \leq 0.05$) *E. coli* and *Ae. aegypti* pupae from the multivariate analysis were derived using backwards selection procedure. All statistical analyses were conducted using SPSS 20.0 (IBM Corp.) and STATA (version 12; STATA Corporation, College Station, TX, USA).

Regarding the two-year surveillance of diarrhoeal disease (**Paper III**), the general information of recorded diarrhoeal cases accounted for in the analysis included their socio-demographic characteristics, type of diarrhoea, diarrhoea treatment and places of the treatment. Obtained diarrhoeal incidence rate was measured as a diarrhoeal episode per 1,000 person-years (Calculating Person-Time 2015). To measure the incidence rate, the actual time in years of each followed household member was used and summed from the beginning of the follow-up date until the occurrence of diarrhoeal event, or until the end of the follow-up time when the event of diarrhoea did not occur. This person-years-at-risk was used as a denominator and the total numbers of diarrhoeal cases in each village derived from two-year record were used as numerators, and then multiply the outcomes with 1000. During the follow-up time, the new born babies together with other people who were not initially registered and have moved into the houses were considered as newly enrolled observations, while those who were lost to the follow up were then excluded from the study. The total period of follow-up time of the people who were lost during the follow up was also used for the calculation of incidence rate in addition to those who remained in the study.

Univariate and multivariate logistic regression models were used to assess the relationship between diarrhoeal disease and risk factors in each village. Backward stepwise selection procedure was used to obtain significant variables ($p \leq 0.05$) from the multivariate analysis. All significant risk factors were used to calculate Population Attributable Fraction (PAF) by using the following formula:

$$\mathbf{PAF} = \frac{\mathbf{Ppop\ x\ (RR-1)}}{\mathbf{Ppop\ x\ (RR-1)+1}},$$

where P_{pop} is the proportion of exposed subjects and RR is the risk or rate ratio associated with the risk factor (Flegal *et al.* 2015).(All statistical analyses were conducted using STATA (version 12; STATA Corporation, College Station, TX, USA).

As for DLI infection surveillance (**Paper IV**), the general information of the patients accounted for in the analysis included personal profile, the number of days living in the village, presence and behavior of taking a nap during a day, modes of dengue protection currently applied by the locals and people’s knowledge on identification of *Aedes* mosquito correctly through a color picture of mosquitos and insects. Distribution of DLI cases over time (month) derived from the two-year follow up was displayed. Similarly to diarrhoea analysis, the univariate and multivariate logistic regression models were used to find an associated relationship between DLI infections and risk factors related to the socio-demographic characteristics and household water management. The number of *Ae. aegypti* pupae per house and pupae per person derived from the entomological survey in each household were used as potential risk factor of DLI infections in this study (Focks *et al*. 2000).

3.4. Ethical considerations

The study was approved by the National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (No. 03/NECHR) on 17 December 2010 and by the Ethical Committee of Phramongkutklao College of Medicine, Bangkok, Thailand (S033h/53) on 21 March 2011. A signed consent form was obtained from the household head of all participating households. Another consent form was also obtained from the individual patient or guardian of DLI infected persons during the two-year follow up.

4. RESULTS

4.1. *Escherichia coli* contamination of household drinking water (Paper I)

The results presented here relate to **Paper I** and **objective 1**. This study showed that the levels of *E. coli* contamination in household drinking water were higher in Laos than in Thailand, and in rural villages compared to suburban villages of both countries (Table 3). The mean *E. coli* concentrations in suburban and rural Laos were 59.2 and 73.5 MPN/100 mL, respectively whereas the corresponding numbers for Thailand were 3.7 MPN and 35.7 MPN/100 mL, respectively. The multivariate analysis showed that the risk factors associated with *E. coli* in household drinking water were site specific; and depended largely on the socio-demographic characteristics, household water management practices and sanitation and hygiene.

A. Effect of sociodemographic characteristics: In all villages (except suburban Thailand), the level of *E. coli* contamination in drinking water was generally high in households with low SES and in houses made of wood (Table 3) (Figure 3C). Drinking water stored in households where houses were made of wood were more likely to be contaminated with *E. coli* than drinking water stored in households with houses made from a combination of cement and wood (Figure 3D) (suburban Laos: IRR: 8.2, CI: 1.9-34.9, rural Thailand: IRR: 2.7, CI: 1.1-6.5) (Table 5).

B. Effect of household water management: The main source of drinking water in the suburban villages of both countries was purchased bottled water whereas rainwater was the main water source in rural villages (Table 3). Drinking water from rain-fed water (Figure 1A) and manually collected rainwater (Figure 1C) were contaminated with *E. coli*. The mean *E. coli* concentration for rain-fed water in suburban Laos was 117.7 MPN/100 mL, while the concentrations for the manually collected rainwater in rural Laos and rural Thailand were 98.4 and 62.3 MPN/100 mL, respectively (Table 3). Purchased bottled water was the least contaminated in both suburban villages (Table 3). However, rain-fed water in rural Laos was less likely to be contaminated with *E. coli* than manually filled containers (IRR: 0.2, CI: 0.1-0.9), but the opposite was found in suburban Thailand (IRR: 33.5, CI: 1.4-771.5). In rural Thailand, manually collected rainwater was nearly 34 times more likely to be contaminated with *E. coli* than non-manually filled containers (IRR: 33.6, CI: 9.2-122.5) (Table 5). In addition to rainwater and purchased bottled water in suburban Laos, borehole water was contaminated (112.2 MPN/100 mL) (Table 3) and almost significantly associated with *E. coli* contamination (IRR: 4.8, CI: 0.9-25.9, p=0.067) (Table 5).

The study found that drinking water stored in jars and bottles were more likely to be contaminated with *E. coli* in suburban Laos, with (IRR: 8.9, CI: 0.9-79.4) and (IRR: 20.7, CI: 4.0-106.0), respectively (Table 5). Similar results were found with the storage of drinking water in jar and buckets in suburban Thailand, with (IRR: 23.0, CI: 2.2-244.4) and (IRR: 4.9, CI: 1.1-22.5), respectively (Table 5). In all villages, although the majority of the containers had lids (80-98%), the containers covered with lids were more likely to be contaminated with *E. coli* in rural Laos (IRR: 5.6, CI: 1.3-24.5) compared with those without lids (Table 5). In Laos, 98% of the containers were cleaned at least biweekly, whereas they were cleaned less frequently in Thailand. In suburban Thailand, drinking water containers that were cleaned less frequently (monthly and longer, up to yearly), were less likely to be contaminated with *E. coli* compared with those that were cleaned more frequently (IRR: 0.01, CI: 0.0-0.6). However, in rural Thailand, containers that were cleaned that monthly and up to yearly period were more likely to be contaminated with *E. coli* than containers that were cleaned more frequently (IRR: 7.9, CI: 2.2-28.5) (Table 5).

C. Effect of sanitation and hygiene: In Thailand, nearly 100% of all households (100% in suburban and 98.4% in rural village) had toilet facilities (Table 2), whereas only 78.2% and 29.8% of the households in suburban and rural village, respectively in Laos had toilets. Households without toilets had higher levels of *E. coli* contamination in their stored drinking water than households with toilets (Table 3). In Laos, the mean *E. coli* concentrations in drinking water in suburban and rural villages without toilets were 145.8 and 90.3 MPN/100 mL, respectively. Drinking water in households without toilets in suburban Laos was around 7 times (IRR: 7.2, CI: 1.6-31.5) more likely to be contaminated with *E. coli* compared with those using pour flush toilets, and in rural Laos they were nearly 17 times (IRR: 16.6, CI: 3.8-65.3) more likely to be contaminated with *E. coli* (Table 5). In Thailand, nearly all households had toilets and the average levels of *E. coli* in stored drinking water was generally lower than in Laos (Table 4). The proportions of households with toilets and handwashing facilities with soap in suburban and rural Laos were also low; 66.1% and 23.5% respectively. In suburban and rural Thailand, the corresponding figures were 84.3% and 98.4% (Table 2).

The households without hand washing facilities with soap near or inside the toilets in Laos had higher *E. coli* concentrations in drinking water than those with soap. However, no associated relationship was found between the handwashing facility and *E. coli* contamination in drinking water.

4.2. *Aedes aegypti* production in household's stored water containers (Paper II)

This section relates to **objective 2** and presented in **Paper II**. The study showed that *Aedes aegypti* pupae positive containers were found in all four study villages. In suburban and rural Thailand, 57% and 47% of the containers were positive for *Ae. aegypti* pupae, respectively. In Laos, the corresponding figures in suburban and rural villages were 54% and 33%. Of these positive containers, the total numbers of *Ae. aegypti* pupae recorded in each village were 1,046 and 1,007 pupae in the Thai villages, and 911 and 558 pupae in the Lao villages, respectively (Tables 3 and 4).

From the multivariate analysis (Table 6), the significant risk factors of *Ae. aegypti* pupae infestations were mostly associated with household water management rather than socio-demographic factors.

A. Effect of socio-demographic characteristics: In suburban Thailand, the significant risk factors of *Ae. aegypti* pupae were associated with respondents who were involved in 'other' occupations (retired, unemployed or student), which are about two times more likely to have *Ae. aegypti* pupae in their homes (IRR: 2.3, CI: 1.1–4.8) compared to farmers' households. In rural Thailand, the corresponding risk factors were involved in 'commercial' occupations (IRR: 0.1, CI: 0.01–0.8) compared to those with agricultural occupations, households with the intermediate (IRR: 9.3, CI: 3.1–28.1) and high SES categories (IRR: 13.2, CI: 4.01–43.3) compared to poor households. In Lao villages, no associated relationship was found between socio-demographic characteristics and *Ae. aegypti* pupae.

B. Effect of household water management: The analysis showed that, in Laos, container type and lid status were found as the only risk factors. Water tanks were more likely to be infested with *Ae. aegypti* pupae compared to other containers in rural Laos (IRR: 5.9, CI: 1.9–19.1), while containers with lids were significantly less likely to be infested with *Ae. aegypti* pupae than those without lids in the suburban Laos (IRR: 0.3, CI: 0.1–0.9). In suburban Thailand, similar outcomes were obtained where the containers with lids were a protective factor (IRR: 0.1, CI: 0.04–0.4). In addition, containers that were cleaned once in more than a week and up to one month were more likely to have *Ae. aegypti* pupae in their homes (IRR: 3.5, CI: 1.9–6.6) compared to containers that were cleaned once a week. In rural Thailand, the significant factors associated with *Ae. aegypti* pupae infestations were; containers that were cleaned once in more than a week and up to one month (IRR: 2.6, CI: 1.3–5.1) compared to those that were cleaned once a week, and containers located outdoors (IRR: 0.2, CI: 0.1–0.5) and in the toilet/bathroom (IRR: 0.4, CI: 0.2–0.9) compared to those located indoors.

4.3. Diarrhoeal disease incidence and risk factors (Paper III)

This section relates to **objective 3**, and is presented in **paper III**. The follow up during 2011-2013of diarrhoeal disease involved 2,007 people; this included 567 and 599 persons from suburban and rural villages in Laos and 402 and 439 persons from Thai villages, respectively. The mean ages of the people followed-up in suburban and rural Laos were 25.7 and 28.1 years; while older people with mean ages 40.2 and 41.6 years, respectively were found in Thailand. Most people in rural villages finished only primary school and were farmers while higher education levels were found in majority of the people in suburban villages (Table 1).

Among the 2,007 individuals, 97 diarrhoeal cases with no deaths were recorded during two years which included 35 and 11 cases in suburban and rural Laos and 12 and 39 cases in Thai villages, respectively (Table 2). In suburban Laos, 63% of the cases were children ≤5 years old, whereas in rural Thailand, 69% of the cases were 15-78 years. The study revealed that diarrhoeal incidence was slightly higher in rural Thailand than in suburban Laos with 46.5 and 31.8 episodes per 1000 person-years, respectively (Table 3). Children under-five years were the most affected age group accounting for up to 180.0 and 170.5 episodes per 1000 person-years in rural Thailand and suburban Laos, respectively. The crude incidence rates of diarrhoea obtained from this study also showed higher prevalence of diarrhoea in Thailand than Laos (Figure 2) as well as from the secondary data of diarrhoea at the district level obtained from the national surveillance system (Figure 4).

The risk factors of diarrhoea found in each village were site specific and mostly related to hygiene and socio-demographic characteristics, while drinking water sources and sanitation facilities were not significant (Table 5).

A. Effect of socio-demographic factors on diarrhoeal disease incidence: Children under-five years compared with other age-cohorts were more likely to have diarrhoea in suburban Laos (OR: 14.9, CI: 6.4-34.4) and rural Thailand (OR: 3.7, CI: 1.7-8.2). The corresponding Population Attributed Fraction (PAF) of diarrhoea cases in children less than five years to diarrhoeal disease in suburban Laos and rural Thailand was 58% and 15%, respectively (Tables 5 and 6).

B. Effect of sanitation and hygiene on diarrhoeal disease incidence: In suburban Laos, diarrhoea was more likely to be recorded in households disposing of children's stool in the open (OR: 3.9, CI: 1.1-15.5) (PAF: 8%) compared to those who rinsed the stool into a toilet. Diarrhoea was also more likely to be recorded in households whose utensils were left unwashed in the kitchens compared to those who washed their utensils (OR: 2.4, CI: 1.1-5.4) (PAF: 11%). In rural Thailand, diarrhoea episodes were more likely to be recorded in households that delayed cleaning of utensils until the next cooking time or next day compared to immediate cleaning (OR: 2.6, CI: 1.1-6.6) (PAF: 53%). In rural Laos and suburban Thailand where the numbers of cases were low, diarrhoeal incidence was associated with the presence of animal/human faeces in the yard (OR: 9.3, CI: 1.2-74.4) (PAF: 80%) and the presence of flies in the kitchen (OR: 12.6, CI: 3.5-45.2) (PAF: 29%), respectively (Tables 5 and 6).

4.4. Dengue-like illness (DLI) incidence and risk factors (Paper IV)

This section is presented in **Paper IV** and relates to **objective 4**. The follow up of DLI was conducted alongside the diarrhoeal disease surveillance as presented in **Paper III**. Overall, 83 cases of DLI were recorded from this surveillance, which included 69 cases in suburban Laos (age range: 4-75 years, mean: 25 years; male:female ratio: 0.8) and 11 in rural Thailand (age range: 6-67 years, mean: 42 years; male:female ratio: 0.2). Only 3 DLI cases were found in rural Laos (age range: 16-65 years, mean: 49 years; and 3 females) and none was found in suburban Thailand. Among 83 cases, 4 (4.8%) were positive for dengue and these included two cases each from suburban Laos and rural Thailand, where DENV serotypes 1 and 2 were responsible for this infection, respectively (Table 1).

Dengue-like illnesses were recorded in both 2011 (34 cases) and 2012 (34 cases) in suburban Laos (Figure 2A), while most of the cases were recorded in 2011 (10 cases) in rural Thailand (Figure 2A). The study found that the incidence of DLI in suburban Laos was higher than in rural Thailand. The crude incidences of DLI reported in each 2011 and 2012 in suburban Laos were 5,996 per 100,000 populations (34 cases out of 567), while only 2,278 (10 cases out of 439) and 228 (only 1 cases out of 439) per 100,000 populations were reported in 2011 and 2012 in rural Thailand, respectively. These findings were in accordance with the district-level secondary data reported in both countries (Figure 2B), where the incidence of dengue during 2010-2013 in Lakhonpheng district (Laos) was at least 3 times higher than dengue in the same periods in Manchakhiri district (Thailand) (Figure 2B).

A. Effect of socio-demographic factors on DLI: The multivariate logistic analysis showed that **(**Table 3**)**, the significant risk factors of DLI were found only in suburban Laos, and associated with age and people's occupation. Those with the age range of 15-20 years were about 5 times more likely to be infected with DLI as compared to those under five years (OR: 4.8, CI: 1.2-19.6) and people who involved in service occupation as well as in "other" occupation (retired and children), where they had 3 times of risk to be infected with DLI if compared to agricultural occupation, respectively.

5. DISCUSSION

5.1. Causal factors of *Escherichia coli* contamination of drinking water

A. Socio-demographic factors: The study found that some socio-demographic characteristics of households were significantly associated with *E. coli* contamination of stored water, especially in suburban Laos and rural Thailand. Stored drinking water found in houses made of wood and mostly inhabited by households with low SES were more likely to be contaminated with *E. coli.* Several studies have shown that low SES limits the capacity of households to access improved water sources, sanitation and hygiene practices, which are key factors for the microbial deterioration of stored drinking water in the household environment (Blakely *et al*. 2005; Bain *et al.* 2014b).

B. Household water management factors: Rainwater was the main source for drinking water and was contaminated with *E. coli* in the rural villages of both countries (Paper I-Table 3). This may be due to unhygienic water handling practices during rainwater collection, transportation and storage as reported for other drinking water sources (WHO 2002; Clasen & Bastable 2003, Wright *et al*. 2004). For instance in Thailand, it is a common practice by household members to dip plastic buckets or hoses instead of using the present faucets to collect water from the rain jars, although these may be large in size (2,000 liters) (Figures 1 and 2 in the thesis). Despite the use of small rain-jars (200 liters) and frequent cleaning on a biweekly basis in rural Laos, drinking water stored in these jars is vulnerable to contamination. The smaller size may easily allow people access when needed (including children); leading to contamination of the manually collected rainwater. A study showed that households with water storage containers without faucets were more likely to report a high incidence of diarrhoea in children (Yeager *et al.* 1991). Consumption of this as drinking water without boiling may in general lead to an increased risk of gastroenteritis.

Drinking water stored in jars and bottles in suburban Laos and jars and buckets in suburban Thailand were significantly more likely to be contaminated with *E. coli* compared with other storage containers (Paper I-Table 5). The water containers in Thailand were cleaned less frequently than those in Laos. Drinking water containers that were cleaned less frequently (monthly and longer, up to yearly) were more likely to be contaminated with *E. coli* than those that were cleaned more frequently in rural Thailand (Paper I-Table 5). This could be due to the frequent use of large rain jars (2,000 liters) in rural Thailand, since large containers are not easy to clean and can lead to deterioration of water quality over a certain time period. However, the opposite was found in suburban Thailand, where the containers that were cleaned with the same frequency (monthly and longer, up to yearly) were less likely to be contaminated with *E. coli.* This could be due to the facts that suburban Thailand had higher SES than other villages, a low room occupancy rate (Paper I-Table 2), and commonly purchased bottled water (Paper I-Table 3). The combination of these characteristics in suburban Thailand may reduce household members' interaction/contact with stored drinking water, thereby reducing the potential for contamination.

The frequent opening of drinking water containers covered with lids may also partly explain that, these were significantly more likely than those without lids to be contaminated with *E. coli* in rural Laos (Paper I-Table 5). The local handling practices are a probable determinant since this contrasts previous studies (Chidavaenzi *et al.* 1998; Mazengia *et al*. 2002), where faecal and total coliform counts were 50% lower in containers covered with lids compared to those without lids. Furthermore, nearly 70% of drinking water in rural Laos comes from manually collected rainwater, which was found to be highly contaminated with *E. coli* (Paper I-Table 3).

C. Sanitation and hygiene factors: Households in Lao villages had a low coverage of improved toilet facilities for the containment of their excreta; and had high levels of *E. coli* in their stored drinking water. The lack of toilet facilities combined with the absence of handwashing facilities may have accounted for the high level of *E. coli* contamination of the stored drinking water in these villages (Paper I-Tables 3 and 5). Improper containment of human excreta as a result of lack of toilet facilities is reported to increase the likelihood of microbial contamination of stored drinking water within the household environment; and increase the risk of diarrhoeal disease transmission (Tumwine *et al.* 2002). This is also evidenced in the present study (Paper III-Table 5).

5.2. Risk factors and their contributions to diarrhoeal disease incidence

Although the study revealed high levels of *E. coli* contamination of stored drinking water, especially in Laos and rural Thailand, the water samples were not collected from the households at the time when diarrhoea cases occurred (out of the study scope). Also, efforts were made to collect stool samples from the cases in order to identify diarrhoea-causing pathogens, but there were difficulties as patients did not want to provide samples. As a result, we could not see the potential link between *E. coli* contamination of stored drinking water and the incidence of diarrhoea in this study, and that other transmission routes may have a more profound effect in these settings. However, other studies found that the presence of *E. coli* (Jensen *et al.* 2004*;* Levy *et al.* 2012;) and *E. coli* concentrations ≥ 10 CFU/100 mL (Brown *et al*. 2008; Gundry *et al*. 2009) may increase the risk of diarrhoea in a household.

As in the systematic review study of Lamberti *et al*. (2012), this study also found a high incidence of diarrhoeal disease episodes in children under five years compared with other age-groups (Paper III-Table 2). Children under five years often have low immunity against most of diarrhoeal disease-causing pathogens particularly in low and middle income countries (Walker *et al.* 2012).

Consistent with the finding of Yeager *et al*. (1991), this study did not find any relationship between the socio-economic status of households and the incidence of diarrhoeal disease. However, this is contrary to the findings made in Brazil and Ghana. In a study assessing the effect of diarrhoeal disease interventions in Brazil, Genser *et al*. (2008) found a significant relationship between the socio-economic status of households and diarrhoeal disease episodes. A similar finding was made by Seidu *et al.* (2013) in relation to diarrhoeal disease incidence in households applying faecal sludge on their farms. In both the Genser *et al.* (2008) and Seidu *et al.* (2013) studies, low socio-economic status increased the likelihood of diarrhoeal disease incidence.

It was shown that those involved in the improper disposal of stools of children were significantly associated with the presence of diarrhoeal disease (Paper III-Table 6). The poor hygiene practices in relation to improper disposal of stool from children have been reported from households of many countries (WSP-UNICEF 2014; 2015a, 2015b) even when latrines are available (Lanata *et al.* 1998; Majorin *et al.* 2014). This unsafe stool disposal was found to increase the odds of diarrhoeal episodes among the children under-five (Mihrete *et al.* 2014; Cronin *et al*. 2016; Bawankule *et al.* 2017). As evidenced in this study, other factors such as kitchen utensils left unwashed and presence of flies in kitchen as well as delayed cleaning of utensils and having animal or human excreta in the yard, were also associated with the occurrence of diarrhoea. This could occur through a direct contact of children with faeces in yards or eating contaminated foodstuffs being transmitted to by the houseflies.

5.3. Causal factors of *Aedes aegypti* production in stored water

A. Socio-demographic factors: In rural Thailand, households with intermediate and high SES categories were significantly more likely to be associated with *Ae. aegypti* pupae infestation (Paper II-Table 6) as compared to poorer households. This may be because the intermediate and rich households had more water containers than poorer ones, thereby providing more breeding sites for *Ae. aegypti*. Mosquito occurrence is however probably site specific and influenced by a set of other factors. An example of contrary findings was in Colombian towns where water containers in rich households were less likely to be infested with *Ae. aegypti* immatures compared to poor ones (Quintero *et al*. 2009).

B. Household water management factors: Containers that were cleaned less frequently (once during a period of a week and up to one month) had significantly higher levels of *Ae. aegypti* pupae infestation in both the suburban and rural villages of Thailand compared to those that were cleaned once a week (Paper II-Table 6). This is associated with the presence of large containers such as jars and tanks, which are difficult to clean. This is consistent with findings made in previous studies in northern Thailand and six other Asian countries where it was found that cleaning of water containers on a weekly could reduce *Ae. aegypti* larvae production (Phuanukoonnon *et al.* 2005; Arunachalam *et al*. 2010). In Laos, no relationship was found between water container cleaning frequency and *Ae. aegypti* pupae infestation (Paper II-Table 6). This may be because the water storage containers in Laos generally being cleaned more frequently than in Thailand (Paper I-Table3 and Paper II-Table 5).

Our study confirmed that the containers with lids were significantly less likely to be infested with *Ae. aegypti* pupae than those without lids especially in the suburban villages of both countries (Paper II-Table 6). This is in agreement with other studies where the containers without lids or partly covered produced more *Ae. aegypti* than those with lids (Koenraadt *et al.* 2006; Tsuzuki *et al.* 2009; Hiscox *et al.* 2013; Quintero *et al*. 2014).

Aedes aegypti pupae production was high in non-drinking water sources, but there were no relationships found between water source and *Ae. aegypti* pupae in this study (Paper II-Table 6). Open large cement tanks, most commonly located in toilets or bathrooms, were the main containers that were used to store non-drinking water. These tanks were found to be the most infested type of breeding site in all villages (Paper II-Tables 3 and 4). A major reason for the infestation of the tanks is that they are large and difficult to clean more frequently thereby providing good breeding sites for *Aedes* mosquitoes. Interventions towards reducing dengue should be directed towards these tanks, as they are predominantly used in many countries of South-east Asia (Tsuda *et al.* 2002; Tsuzuki *et al*. 2009; Dada *et al.* 2013).

The study also found that the larvicide temephos was not commonly used for the control of *Aedes* larvae. In Laos, only 22% of suburban containers and 6% of rural ones contained temephos. In Thailand, it was even less used as temephos was found in only one percent of containers in the rural village (Tables 3 and 4). Temephos was thus inconsistently used and not an effective dengue control intervention in these settings. In addition to improper use, the lack of use could be due to distribution problems and to perceptions of temephos being a harmful chemical as was also concluded from a study in northeastern Thailand (Phuanukoonnon *et al*. 2006). Indiscriminate use of temephos can also lead to insecticide resistance as identified in some parts of Thailand (Ponlawat *et al.* 2005; Poupardin *et al.* 2014).

5.4. Risk factors of dengue-like illness

Household members within the age cohort of 15-20 years were more likely to be affected by DLI in suburban Laos as compared to lower age groups. This was also the case in a large dengue outbreak in Laos during 2010, where cases were mainly found in the age group of 10 to 19 years (Khampapongpane *et al*. 2014). In a review of dengue conducted in Thailand during 2000-2011 the highest incidence rates were among people aged between 10-14 years old (Limkittikul *et al*. 2014).

According to a previous study, people with higher education (secondary or higher educational degrees) were more likely to be infected by dengue (Koyadun *et al.* 2012), which was also the case in this study (Paper IV, Table 4). This may relate to travel or work patterns outside their residential setting thus increasing their chances of contracting dengue infections compared to those with lower educational levels. Human movement as a result of socio-economic development is a known factor favoring the spread of dengue and other vector-borne diseases (Maidana and Yang 2008; Adams and Kapan 2009; Stoddard *et al.* 2009).

The number of pupae per person was higher than a proposed dengue transmission threshold of between 0.5-1.5 pupae per person (Focks *et al.* 2000) (Paper IV-Table 1). Pupal indices are probably better indicators of dengue transmission and more accurate than the traditional *Stegomyia* indices (House index, Container index, Breteau index) (Focks & Chadee 1997; World Health Organization, 2000; Bowman *et al.* 2014). However, the high pupae per person were not significantly associated with DLI infections (Paper IV-Table 3). This was also the case in a study conducted in the Republic of Palau, where DLI infections were not associated with the pupal index instead the households reported with DLI were significantly more likely to be associated with potential breeding sites of mosquitoes than those without (Umezaki *et al.* 2007). A systematic review on the correlation between vector indices and dengue transmission made by Bowman *et al.* (2014) found no robust relationships to predict dengue outbreaks. Further studies are therefore needed to identify more reliable indices.

6. CONCLUSIONS

The levels of *E. coli* contamination found in all study sites were above the WHO drinking-water quality guidelines of zero *E. coli*/100 mL (WHO 2006). The study revealed that *E. coli* contamination in household drinking water (**Paper I**) was more likely to be associated with household water management practices and sanitation (lackof toilet facilities), especially in Laos, but less associated with socio-demographic characteristics.

According to the follow up in these settings, diarrhoeal incidence remains a problem for children under-five years of age and more prevalent in this study **(Paper III)** compared to the official data from the national surveillance system in both countries. The risk factors in relation to hygiene and socio-demography were mainly responsible for the diarrhoeal transmission in these settings. Health education for appropriate treatment of stored drinking water (e.g. boiling) prior to drinking can provide a significant barrier against diarrhoeal disease incidence in households with poor water quality. In Laos, interventions related to the provision of improved toilet facilities have the potential of not only improving the quality of stored drinking water but also controlling against diarrhoea related to improper disposal of the baby stool. Furthermore, immediate cleaning of utensils after eating or cooking is also important for the reduction of diarrhoea in both Laos and Thailand.

Household water management rather than socio-demographic factors were more likely to be associated with *Ae. aegypti* infestation of water containers. Most of the household water storage containers in all study villages had no lids, contained no temephos larvicide and were cleaned less frequently, thereby providing mosquito breeding suitable sites with high *Ae. aegypti* pupae infestation rates and a risk for dengue transmission (**Paper II**). The incidence rate of DLI was high in Laos than in Thailand (**Paper IV**) and higher in this study compared to the dengue data from the national surveillance system in both countries. Among the positive cases, DENV serotype 1 and serotype 2 were detected in the suburban village of Laos and in the rural village of Thailand, respectively. The circulation of these common serotypes in these settings can lead to potential dengue outbreaks at any time. The study findings stress the need for continuous surveillance to control *Ae. aegypti* in household water storage containers. The vector spreads not only dengue but also chikungunya and Zika viruses. This indicates the need for expansion of surveillance programs to incorporate other febrile illnesses such as chikungunya and Zika, which will have significant impact on early detection of these diseases and subsequent prevention of outbreaks.

To achieve significant reduction in the control of *Ae. aegypti* production in household water containers in these settings, health messages should promote proper use and maintenance of tightly fitted lids, weekly cleaning of water containers especially the smaller ones, as well as adding temephos in water-holding containers such as tanks and jars, which were the most infested containers. Furthermore, adult mosquito control must be considered in an integrated vector management strategy. Although compliance is always an issue when it comes to mosquito control, community participation will be key to the success of any selected control measure.

7. STUDY LIMITATIONS AND FURTHER RESEARCH

The study results presented in **Papers I and II**, would have been stronger if water quality and entomological surveys had been repeated. However, both time and manpower are limiting factors. Risk factors of faecal contamination in drinking water and *Ae. aegypti* infestation in stored water containers related to household water management may further vary between seasons. The number of rainwater storage containers in the wet season may provide more breeding sites for dengue vector production as well as larger variabilities in the levels of *E. coli* contamination. An additional limitation is the lower than expected numbers of diarrhoeal disease cases where the relative impact of the drinking of contaminated water compared to other sources of contamination and transmission could not be revealed. A larger number would probably have given a further identification of microbiological etiological agents in stool of the cases (**Paper III**).

As aforementioned, only four cases were confirmed for positive dengue, where DENV serotype 1 and serotype 2 were detected in Laos and Thailand, respectively (**Paper IV**). These serotypes were also identified in higher proportions during the dengue outbreaks in 2010 in both Laos (Khampapongpane *et al.* 2014) and Thailand (Limkittikul *et al.* 2014). However, other arboviral infections such as chikungunya or Zika could have been responsible for undetected dengue in this study, because they have similar clinical manifestations and the same transmission vectors (World Health Organization, 2009). Additionally a higher incidence of dengue cases would have given more in-depth identification results both in relation to dengue and to other *Aedes* transmitted disease cases. A recent outbreak of DLI in Pernambuco of Brazil (2015) showed that 11.7% and 1.3% of the cases were confirmed dengue and chikungunya, respectively, with Zika accounting for 40.2% (Pessôa *et al*. 2016). A cohort study conducted in Indonesia, Malaysia, Philippines, Thailand and Vietnam found that, among 1,500 healthy children aged 2-14 years, the most common causes of acute febrile illness (≥38°C for ≥2 days) were chikungunya, scrub typhus and dengue (Capeding *et al.* 2013). In addition, other infections could also be responsible for the observed DLI in these settings, for example scrub typhus, influenza, Japanese encephalitis and leptospirosis, which are common causes of acute febrile illnesses in Laos (Mayxay *et al.* 2013; 2015). Leptospirosis and scrub typhus have been shown to be major causes of acute febrile illnesses in rural Thailand as well (Suttinont *et al*. 2006).

Another reason for the low numbers of confirmed dengue cases could be due to a problem of poor sample preservation caused by intermittent electricity shut-downs, which could affect the temperature of stored dengue samples in -20 ^{o}C. This is a common limitation in epidemiological studies in developing countries.

During the two-year follow up survey, there were few cases of diarrhoea and DLI recorded in the rural village of Laos and in the suburban village of Thailand. Low motivation of village health volunteers to collect the cases as well as un-willingness to give information of the household members may have affected the result with fewer reported numbers than expected. Other factors could be due to the fact that suburban Thailand is recognized as the richest village compared to the other ones in this study, resulting in people would be able to access other resources to protect themselves from getting diarrhoea and dengue infections, such as purchased bottled water and dengue protection tools (air conditioners, insecticide spray etc.). In contrast, rural Laos is the poorest and the only village that has no health facility; therefore people in this village may have a poor concern for their health status as they may get used to living without seeking health care, especially if the symptoms or illnesses are not severe enough.

Further research to be considered:

1. Temephos resistance in Lao villages, the need for evaluation of present dengue control.
2. Identification of chikungunya virus and Zika among dengue-like illnesses in suburban and rural villages of Laos.
3. Control of immature *Aedes aegypti* with a focus on key breeding sites (jars and tanks) of the household water storage containers.
4. Create the model households on dengue immature control with the support of village health volunteers in Laos.
5. Etiological causes of diarrhoeal disease in relation to drinking untreated rain water in Laos and Thailand.

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PAPER I

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Assessing factors of *E. coli* contamination of household drinking water in suburban and rural Laos and Thailand

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ABSTRACT

Drinking water (DW) can serve as a route for disease transmission if not properly managed. The study assessed the effect of different factors on *E. coli* quantities in DW in household water storage containers in suburban and rural villages in Laos and Thailand. Higher *E. coli* concentrations in DW were found in Laos compared to Thailand, especially in households without toilets (in Laos) and in rural rather than in suburban villages. In suburban Laos, house material, storage container types and lack of toilets were significantly associated with *E. coli* contamination of DW, whereas in rural Laos, corresponding significant factors were rain-fed water, containers with lids and lack of toilets. In suburban Thailand, rain-fed water, storage container types and container cleaning frequency were significantly associated with DW contamination, while house materials, manually collected rainwater and container cleaning frequency were associated with contamination in rural Thailand. Socio-demographic characteristics were less associated with *E. coli* contamination of DW in this study. Treatment of household stored water (e.g. boiling), regular cleaning of rain jars as well as the provision of household toilets especially in Laos can provide barriers against *E. coli* contamination of DW.

Key words | *Escherichia coli*, household drinking water, Laos, socio-demographic, Thailand

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INTRODUCTION

Over the last decade, the diarrhoeal mortality among children under five has decreased globally from 1.2 million (in 2000) to 760,000 (in 2011), but 90% of these child deaths are still linked to water, sanitation and hygiene (UNICEF 2013). In South-east Asia, 363,904 diarrhoeal deaths of all ages were estimated to be linked with inadequate water, sanitation and hygiene, which constitute 56% of diarrhoeal diseases in this region (Prüss-Ustün *et al.* 2014).

Storage of drinking water (DW) is a common practice in many countries where access to DW is either not available within the home environment or, if available, flows intermittently. DW can serve as a source of diarrhoeal disease transmission if not properly managed (WHO 2002). Among the causes of diarrhoeal disease incidence, the storage of DW within the household environment remains a significant risk factor (Roberts *et al.* 2001; Günther & Schipper 2013). All

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pathogens of viral, bacterial, parasitic and protozoan origins, implicated in gastroenteric disease outbreaks, may be found in the source water if contaminated, or introduced into the stored water due to poor handling.

In Laos and Thailand, many households have access to tap water, but prefer rainwater because the taste is considered better (Pinfold *et al.* 1993). Rainwater is collected during the wet season and stored throughout the year. Large cement jars (>2,000 liters), introduced by governmental programs in the 1980's, are the main water storage containers in Thailand. Smaller jars of 200 liters or less and plastic buckets are more common in Laos, but are also found in Thailand (Pinfold *et al.* 1993; Dada *et al.* 2013; Hiscox *et al.* 2013). A review on household DW in developing countries showed that stored water is often of a worse microbial quality than water from the source (Wright *et al.* 2004). Storage even for a short time can lead to microbial contamination of good-quality DW due to unhygienic handling. The stored DW in both Laos and Thailand was previously shown to partly be affected by microbial contamination with *Escherichia coli* (*E. coli*) (Dada *et al.* 2013) and did not comply with the WHO guideline of zero *E. coli*/100 mL (WHO 2006). A study conducted in Cambodia found a significant relationship between diarrhoeal disease and *E. coli* levels of ≥11 CFU/100 mL in DW compared with a reference *E. coli* level of <1 CFU/100 mL (Brown *et al.* 2008). Similar findings were made in rural households in South Africa and Zimbabwe where diarrhoeal disease was associated with *E. coli* levels of ≥10 CFU/100 mL in household DW collected from a communal source (Gundry *et al.* 2009). Other studies have found significant relationships between diarrhoeal disease incidence and the presence of *E. coli* in household DW (Jensen *et al.* 2004; Levy *et al.* 2012).

Several factors affect the quality of stored DW in the household environment. These factors include water collection and storage (Jensen *et al.* 2002; Clasen & Andrew 2003); hygiene and handling practices (Gundry *et al.* 2006; Eshcol *et al.* 2009; Rufener *et al.* 2010); as well as contamination at the source (Taneja *et al.* 2011; Too *et al.* 2016). There are few studies in South-east Asia especially in Laos and Thailand on the main drivers of microbial contamination of stored DW in the household environment. However, for the development of cost-effective interventions, it is critical that factors contributing to the microbial contamination of DW in the household environment are identified. The main aim of this study is to assess these factors and their contribution to the microbial contamination of stored DW in suburban and rural villages in Laos and Thailand.

MATERIALS AND METHODS

Study areas

This study was conducted from February to April 2011 in Manchakhiri district, Khon Kaen province in northeastern Thailand, and from May to June 2011 in Lakhonpheng district, Salavan province in southern Laos (Figure 1). Using existing health data and in consultation with each country's public health departments, one suburban and one rural village per country were selected based on previously described criteria (Dada *et al.* 2013).

Study design and data collection

The study was a cross-sectional survey involving household interviews, observations and water sampling. A systematic random sampling procedure was used in the selection of study households from a total of 215 and 128 households in the suburban and rural villages in Laos, and from a total of 139 and 272 households in the suburban and rural villages in Thailand, respectively. This resulted in the inclusion of 121 and 114 households from suburban and rural Laos, and 117 and 121 households from suburban and rural Thailand, respectively. General characteristics of the households were obtained through interviews using semi-structured questionnaires administered to household heads. Information collected included socio-demographic characteristics (Tables 1 and 2), DW sources (e.g. rain-fed water (rainwater that is collected directly from the rooftop, through the roof-connected tube or from a metal roofing sheet), manually collected rainwater (rainwater that is collected manually from large containers), purchased bottled water, and borehole water), water management practices, sanitation facilities and hygiene. This was further supplemented by observations to ascertain the types of toilet facilities that were in use, presence of soap at handwashing

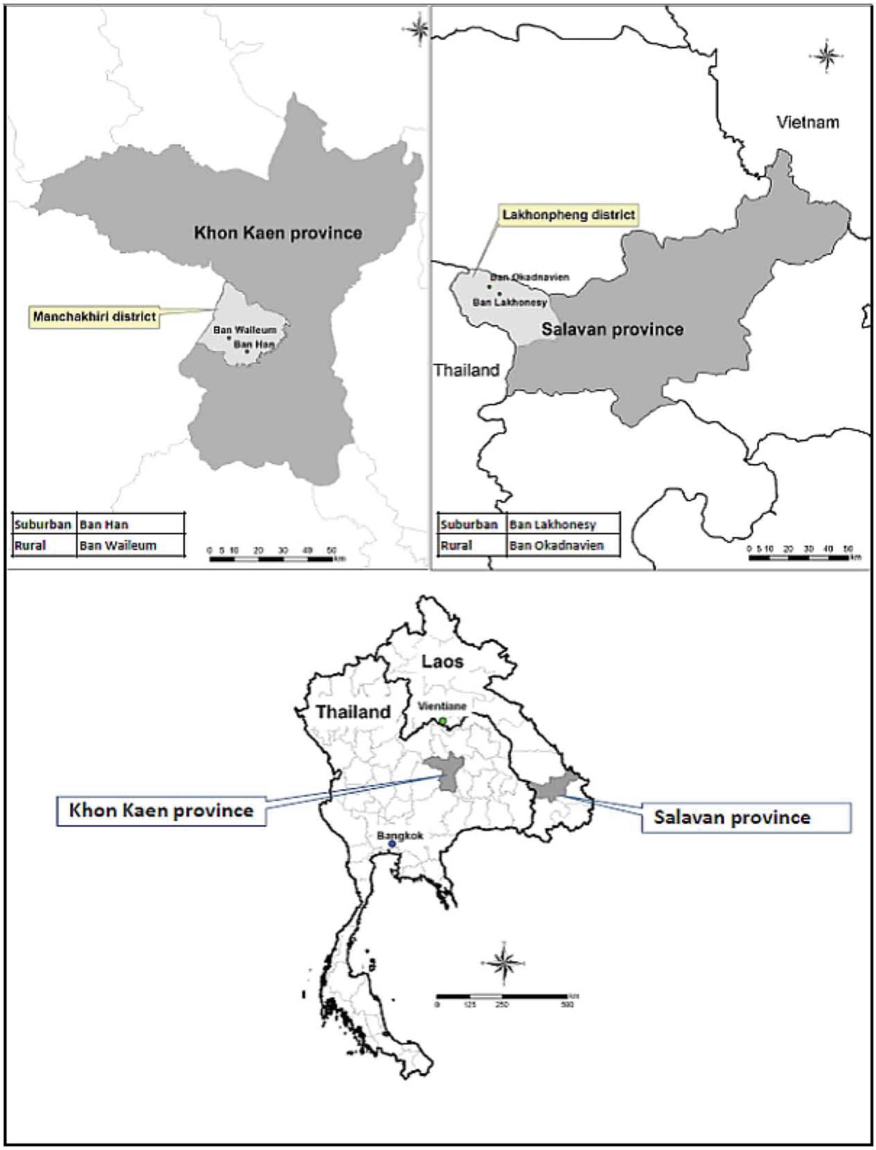

Figure 1 | Study villages in Laos and Thailand (Dada et al. 2013).

facility, types of water storage containers, presence or absence of a secured lid on the water storage containers and mode of collecting water to drink. The interviews and observational surveys were conducted together with trained field staff (village health volunteers). The DW samples were collected from a total of 139 and 145 water containers in

Table 1 | Variables used in the wealth status ranking

Variables	Options/Values
House material	Cement/wooden/cement-wood
House floor material	Cement/wooden/cement-wood
Room occupancy rate	>2.5 persons per habitable room/ ≤ 2.5 persons per habitable room
Ownership of durable assets	Mobile phone/cell phone/TV/radio/ refrigerator/car/motorcycle/bicycle
Affordability of bottled water	Can afford/cannot afford
Ownership of toilet facility	Yes/No
Ownership of flush toilet	Yes/No
Ownership of pour flush toilet	Yes/No

suburban and rural Laos. In Thailand, 178 and 268 DW samples were collected from the suburban and rural villages respectively. Samples were collected using sterile 100 mL Whirl-Pak bags, put on ice and transported to a field laboratory where they were analyzed for *E. coli* within 24 hours after sampling. *E. coli* analysis was done using Colisure-Quantitray/2000 method (Colisure® WCLS2001, IDEXX laboratories, Inc., Westbrook, USA). Details of the *E. coli* analysis and identification procedures are described in Dada *et al.* (2013). *E. coli* results were expressed as most probable number (MPN)/100 mL.

E. coli was chosen as a faecal indicator in this study because it is easy and less costly to analyze; and is identified as the most suitable indicator of faecal contamination in DW. Also, it is generally considered as a reliable indicator for the presence or absence of other pathogenic bacteria such as *Salmonella*, *Shigella* and *Campylobacter spp.* (WHO 2017). Although thermotolerant coliforms are also recommended and used as alternative group of faecal indicator organisms, a review showed that *E. coli,* rather than thermotolerant coliforms, in household DW was significantly associated with diarrhoea (Gruber *et al.* 2014).

Data analysis

Descriptive analysis was undertaken to examine the statistical distribution (frequency, percentage, central tendency and rate) of factors related to socio-demographic characteristics, DW, water management, sanitation and hygiene. The mean, minimum and maximum of *E. coli* (MPN/100 mL) concentration related to all study factors were displayed. Households were ranked into rich, intermediate and poor ones using principal components analysis and based on group weighted mean scores (Vyas & Kumaranayake 2006). The variables used in the wealth status ranking are presented in Table 1. A univariate analysis was undertaken to assess the independent effect of these factors on the occurrence of *E. coli* in the household DW. Regardless of significance, all factors included in the univariate analysis were entered into a zero-inflated negative binomial (ZINB) multivariate model to assess the effect of multiple factors on the occurrence of *E. coli* in the DW in households. The ZINB model was used to account for over-dispersion and excess zeroes in *E. coli* values. From the multivariate ZINB analysis, incidence rate ratios (IRR) and their 95% confidence intervals were obtained. The IRRs of factors significantly affecting ($p \leq 0.05$) *E. coli* concentration in the DW from the univariate and multivariate analysis are presented. All statistical analyses were conducted using STATA (version 12; STATA Corporation, College Station, TX, USA).

RESULTS

Socio-demographic characteristics of households

The socio-demographic characteristics of the study households are presented in Table 2. Over 90% of the household members in both countries were literate and the main occupation was agriculture, especially in the rural villages in both countries (over 94%). Households in Thailand were wealthier than in Laos. In Thailand, most houses were made of cement-wood (70% in suburban and 61% in rural village); while in Laos, the houses were mainly made of wood alone (42% in suburban and 85% in rural village).

DW sources, management practices and levels of *E. coli* contamination

The main source of DW in both suburban Thailand and Laos was purchased bottled water (Combined Table 3a and 3b)

Table 2 | Household characteristics in suburban and rural villages in Laos and Thailand

		Laos		Thailand	
		Suburban	Rural	Suburban	Rural
No. of households		121	114	117	121
Gender	Male	40 (33.1)	47 (41.2)	59 (50.4)	88 (72.7)
	Female	81 (66.9)	67 (58.8)	58 (49.6)	33 (27.3)
Literate	Yes	110 (90.9)	104 (91.2)	114 (97.4)	117 (96.7)
	No	11 (9.1)	10 (8.8)	3 (2.6)	4 (3.3)
Occupation	Agriculture	63 (52.1)	108 (94.7)	28 (23.9)	116 (95.8)
	Commerce	12 (9.9)	3 (2.6)	29 (24.8)	1 (0.8)
	Service	30 (24.8)	3 (2.6)	9 (7.7)	2 (1.7)
	Other[a]	16 (13.2)	0	51 (43.6)	2 (1.7)
Room occupancy rate	≤2.5 persons/room	61 (50.4)	71 (62.3)	96 (82.1)	91 (75.2)
	>2.5 persons/room	60 (49.6)	43 (37.7)	21 (17.9)	30 (24.8)
Wealth status	Poor	39 (32.2)	92 (80.7)	5 (4.3)	19 (15.7)
	Intermediate	49 (40.5)	16 (14.0)	36 (30.8)	65 (53.7)
	Rich	33 (27.3)	6 (5.3)	76 (64.9)	37 (30.6)
Housing material	Cement and wood	45 (37.2)	16 (14.0)	82 (70.1)	74 (61.2)
	Cement	25 (20.7)	1 (0.9)	23 (19.7)	15 (12.4)
	Wood	51 (42.1)	97 (85.1)	12 (10.2)	32 (26.4)
Floor material	Cement and wood	28 (23.1)	14 (12.3)	76 (65.0)	62 (51.2)
	Cement	37 (30.6)	6 (5.3)	26 (22.2)	26 (21.5)
	Wood	53 (43.8)	94 (82.5)	14 (11.9)	32 (26.5)
	Ground	3 (2.5)	0	1 (0.9)	1 (0.8)
Mode of collecting water to drink	Pouring into cup	79 (65.3)	75 (65.8)	49 (41.9)	39 (32.2)
	Scooping into cup	42 (34.7)	39 (34.2)	68 (58.1)	82 (67.8)
Sanitation facility	Pour flush toilet	77 (63.6)	25 (21.9)	99 (84.6)	119 (98.3)
	Flush toilet	7 (5.8)	1 (0.9)	18 (15.4)	0
	No toilet	37 (30.6)	88 (77.2)	0	2 (1.7)
Hand washing facility with soap near or inside the toilet					
	Yes	80 (66.1)	26 (22.8)	98 (83.8)	119 (98.3)
	No	41 (33.9)	88 (77.2)	19 (16.2)	2 (1.7)

Percentages in parentheses.
[a]Retired, unemployed and student.

whereas rainwater collection was the most common both in rural Thailand (directly or drawn from the collection jars) and in rural Laos (manually drawn from the collection jars).

Generally, the highest levels of *E. coli* contamination in DW were recorded in the rural villages compared to the suburban villages (Table 3a and 3b). The levels of *E. coli* contamination was higher in Laos than in Thailand especially in rain-fed water (mean: 117.7 MPN/100 mL, min.: 0, max.: 1,986 MPN/100 mL) in suburban Laos and manually collected rainwater in rural Laos (98.4 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL) (Table 3a). The suburban village in Thailand had the lowest mean *E. coli* contamination level (3.7 MPN/100 mL, min.: 0, max.: 105 MPN/100 mL) compared to all other sites (Figure 2). Purchased bottled water and borehole (not available in Thailand and rural Laos) were the least contaminated across study villages except in suburban Laos, with a mean of 112.2 MPN/100 mL, min.: 0, and max.: 2,420 MPN/100 mL (Table 3a).

Although the majority of the containers in all villages had lids (80–98%) they were unprotected from contamination especially in both rural villages. The concentration of *E. coli* was high in stored DW in households that collected water by scooping compared to those that poured

Table 3 | Contamination of *E. coli* in stored DW (*E. coli* MPN/100 mL) related to socio-demographic, DW sources, household water management, sanitation and hygiene in suburban and rural villages in Laos and Thailand

		Suburban		Rural	
		n	Mean (min-max)	n	Mean (min-max)
a. Laos					
No. of containers		139		145	
Socio-demography					
Study locations		139	59.2 (0–2,420)	145	73.5 (0–2,420)
Literacy	Yes	124	61.9 (0–2,420)	130	63.3 (0–2,420)
	No	15	37.3 (0–248)	15	161.7 (0–2,420)
Occupation	Agriculture	77	90.4 (0–2,420)	139	76.6 (0–2,420)
	Commerce	12	7.4 (0–66)	3	2.3 (0–4)
	Service	33	33.8 (0–921)	3	0.7 (0–2)
	Other[a]	17	4.0 (0–27)	0	
Room occupancy rate	≤2.5 persons/room	72	36.6 (0–1,986)	93	104.6 (0–2,420)
	>2.5 persons/room	67	83.5 (0–2,420)	52	17.9 (0.222)
Wealth status	Poor	49	141.1 (0–2,420)	120	85.8 (0–2,420)
	Intermediate	57	22.5 (0–921)	19	15.7 (0–179)
	Rich	33	1.1 (0–20)	6	11.5 (0–53)
Housing material	Cement and wood	52	4.3 (0–147)	19	19.4 (0–179)
	Cement	26	3.1 (0–72)	1	0
	Wood	61	129.9 (0–2,420)	125	82.3 (0–2,420)
Sources of DW (most used)	Rain-fed	19	117.7 (0–1,986)	34	18.9 (0–222)
	Manually collected rain	22	13.5 (0–248)	101	98.4 (0–2,420)
	Purchased bottled water	70	21.1 (0–921)	10	7.6 (0–53)
	Borehole	25	112.2 (0–2,420)	0	
Household water management					
Types of DW storage containers (most used)	Jar	26	145.7 (0–1,986)	46	27.6 (0–548)
	Bucket	33	11.2 (0–147)	44	67.8 (0–2,420)
	Bottle	66	59.5 (0–2,420)	30	33.4 (0–579)
	Jug	8	0.3 (0–2)	15	179.9 (0–2,420)
	Others	3	1.3 (0–4)	6	450.8 (0–2,420)
Lid status	Without lid	4	0	29	11.2 (0–107)
	With lid	135	60.9 (0–2,420)	116	89.1 (0–2,420)
Mode of collecting water to drink	Pouring into cup	84	19.6 (0–921)	98	64.6 (0–2,420)
	Scooping into cup	55	119.7 (0–2,420)	47	92.2 (0–2,420)
Frequency of container cleaning	At least biweekly	137	60.1 (0–2,420)	142	75.1 (0–2,420)
	Monthly	0		2	0.5 (0–1)
	>Monthly to yearly	2	0	1	1.0 (1-1)
Sanitation facility	Pour flush toilet	86	17.5 (0–921)	28	6.4 (0–55)
	Flush toilet	7	3.0 (0–10)	1	3.0 (3-3)
	No toilet	46	145.8 (0–2,420)	116	90.3 (0–2,420)
Hygiene					
Hand washing facility with soap near or inside the toilet	No	52	70.1 (0–1,986)	117	88.5 (0–2,420)
	Yes	87	52.7 (0–2,420)	28	10.9 (0–179)
b. Thailand					
No. of containers		178		268	

(*continued*)

Table 3 | continued

		Suburban		Rural	
		n	Mean (min-max)	n	Mean (min-max)
Socio-demography					
Study locations		178	3.7 (0–105)	268	35.7 (0–2,420)
Literacy	Yes	175	3.6 (0–105)	258	37 (0–2,420)
	No	3	7 (0–105)	10	0.4 (0–4)
Occupation	Agriculture	45	2.1 (0–23)	255	37.4 (0–2,420)
	Commerce	36	5.5 (0–83)	2	5.5 (5–6)
	Service	14	1.1 (0–13)	3	1.7 (0–4)
	Other[a]	83	4.2 (0–105)	8	0.5 (0–4)
Room occupancy rate	≤2.5 persons/room	146	3.6 (1–105)	205	41.6 (0–2,420)
	>2.5 persons/room	32	4.1 (0–46)	63	16.3 (0–756)
Wealth status	Poor	8	2.0 (0–16)	42	88.4 (0–2,420)
	Intermediate	57	5.5 (0–105)	143	35.8 (0–2,420)
	Rich	113	2.9 (0–83)	83	8.7 (0–228)
Housing material	Cement and wood	127	3.1 (0–105)	157	9.8 (0–488)
	Cement	32	4.8 (0–83)	39	64.7 (0–2,420)
	Wood	19	6.1 (0–46)	72	76.3 (0–2,420)
Sources of DW (most used)	Rain-fed	49	3.8 (0–105)	122	5.3 (0–79)
	Manually collected rain	46	3.3 (0–34)	143	62.3 (0–2,420)
	Purchased bottled water	78	3.9 (0–83)	2	0.5 (0–1)
	Borehole	0		0	
Household water management					
Types of DW storage containers (most used)	Jar	57	3.2 (0–105)	188	25.9 (0–2,420)
	Bucket	70	6.4 (0–83)	76	61.8 (0–2,420)
	Bottle	38	0.4 (0–12)	1	0
	Jug	4	0.8 (0–3)	1	1 (1-1)
	Others	7	0.4 (0–2)	1	1 (1-1)
Lid status	Without lid	2	0	4	2.5 (0–8)
	With lid	176	3.7 (0–105)	264	36.2 (0–2,420)
Mode of collecting water to drink	Pouring into cup	65	2.9 (0–105)	76	47 (0–2,420)
	Scooping into cup	113	4.1 (0–83)	192	31.2 (0–2,420)
Frequency of container cleaning	At least biweekly	128	3.6 (0–83)	136	29.7 (0–866)
	Monthly	7	1.9 (0–13)	13	189.6 (0–2,420)
	>Monthly to yearly	43	4.1 (0–105)	119	25.7 (0–2,420)
Sanitation facility	Pour flush toilet	151	3.4 (0–83)	265	36.1 (0–2,420)
	Flush toilet	27	5.4 (0–105)	0	
	No toilet	0		3	0
Hygiene					
Hand washing facility with soap near or inside the toilet	No	33	2.8 (0–46)	4	0.5 (0–2)
	Yes	145	3.9 (0–105)	264	36.2 (0–2,420)

Min-max: minimum-maximum.
[a]Retired, unemployed and student.

from containers to drink, except in rural Thailand (Table 3b). Nearly all containers in Laos (98%) were reported to be cleaned at least biweekly. In Thailand, however, containers were cleaned less frequently; 30% of suburban containers and 50% of rural containers were cleaned monthly or less frequently (Table 3b).

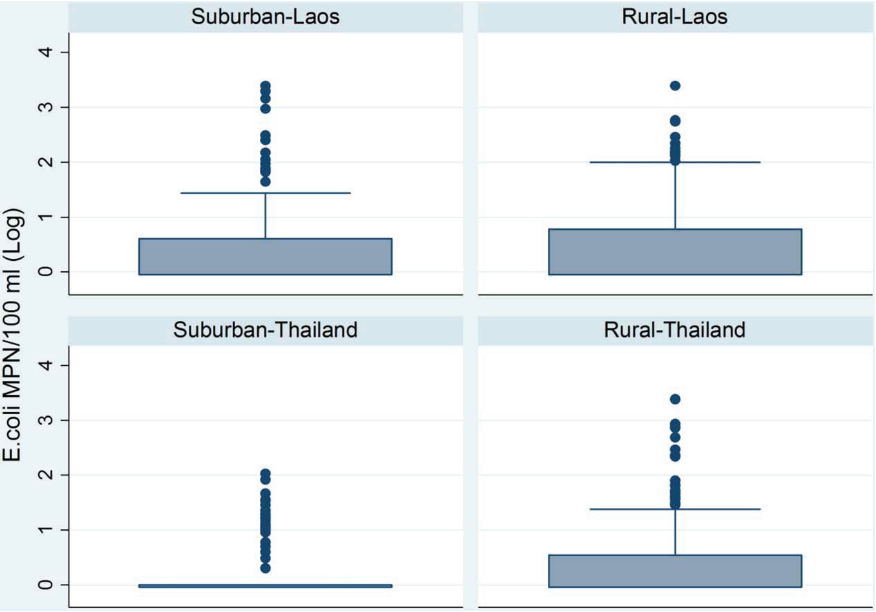

Figure 2 | Log *E. coli* Most Probable Number (MPN)/100 mL in household DW in suburban and rural villages of Laos and Thailand.

Types of DW containers

Bottles were the most commonly used DW containers in suburban Laos, followed by buckets, while in the rural village jars and buckets were used equally (Table 3a). In Thailand, generally, jars and buckets were the most commonly used DW containers, with jars being the most predominant in the rural village (Table 3b). Jugs and containers grouped as 'other' were more common in Laos than in Thailand, but were the least used across all sites (Table 3a and 3b). Of the most common containers, jars in suburban Laos had the highest mean contamination levels (145.7 MPN/100 mL, min.: 0, max.: 1,986 MPN/100 mL), followed by buckets in rural Laos (67.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL), and buckets in rural Thailand (61.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL). However those less commonly used, i.e. jugs and 'other' had the highest mean *E. coli* contamination level than all of the containers inspected, 179.9 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL and 450.8 MPN/100 mL, min.: 0, max.: 2,420 MPN/100 mL, respectively (Table 3a).

Sanitation and hygiene

In Laos, 77.2% of the households in rural villages had no toilet facilities compared with 30.6% in suburban villages. In Thailand, nearly all households (100% in suburban and 98.3% in rural village) had toilet facilities (Table 2). The proportions of households with toilets and handwashing facilities with soap in the suburban and rural Laos were 66.1% and 22.8%, respectively. In suburban and rural Thailand, the proportion of households with toilets, in addition to handwashing facilities was 83.8% and 98.3% respectively.

Households without toilets had higher levels of *E. coli* contamination in their stored DW than households with toilets (Table 3a). In Laos, the mean *E. coli* concentrations in DW in suburban and rural villages without toilets were 145.8 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL) and 90.3 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL), respectively. In Thailand, nearly all households had toilets and the average levels of *E. coli* in stored DW was generally lower than in Laos (Table 3b). In addition, households without hand washing facility with soap near or inside the toilet in Laos had higher *E. coli* concentrations in DW than those with soap. In suburban Laos the mean *E. coli* concentrations in DW for households without hand washing facility with soap and those households with handwashing facilities was 70.1 MPN/100 mL (min.: 0, max.: 1,986 MPN/100 mL) and 52.7 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL). In rural Laos, the mean concentration of *E. coli* in drinking was 88.5 MPN/100 mL (min.: 0, max.: 2,420 MPN/100 mL) for households without handwashing facilities and 10.9 MPN/100 mL (min.: 0, max.: 179 MPN/100 mL) for households with handwashing facilities (Table 3a).

Univariate analysis

Table 4 presents the significant factors associated with the occurrence of *E. coli* in stored DW from the univariate analysis. In suburban Thailand, none of the factors were significantly associated with the occurrence of *E. coli* in DW. The occupation of household heads was significantly associated with the occurrence of *E. coli* in DW in both rural Laos and Thailand (Table 4). Generally, DW in poor wealth status households was more likely to be contaminated with *E. coli* than DW in higher wealth status households (intermediate and rich). In rural Laos, DW in the intermediate wealthy households was less likely to be contaminated with *E. coli* than DW in the poor households (IRR: 0.2; CI: [0.0–0.9]; $p = 0.045$). Similarly, DW in rich households were less likely to be contaminated with *E. coli* than DW from poor households in suburban Laos (IRR: 0.01; CI: [0.0–0.1]; $p < 0.001$) and rural Thailand (IRR: 0.1; CI: [0.0–0.4]; $p = 0.001$). In rural Laos, households with more than 2.5 persons per habitable room were less likely to have *E. coli* contaminated DW compared to those with less than 2.5 persons per habitable room (IRR: 0.1; CI: [0.0–0.4]; $p < 0.001$). In rural Thailand, *E. coli* contamination of stored DW was associated with the type of housing material. In this regard, DW stored in houses made of cement were more likely to be contaminated with *E. coli* than DW stored in houses made from both wood and cement (IRR: 10.3; CI: [2.7–40.3]; $p = 0.001$). Similarly, DW stored in houses constructed with only wood were more likely to be contaminated with *E. coli* than water stored in houses made from both wood and cement (IRR: 9.0; CI: [3.5–23.3]; $p < 0.001$).

With respect to DW sources, some water sources in both rural villages were significantly associated with the occurrence of *E. coli* in DW. In rural Laos, rain-fed water and purchased bottled water were significantly less likely to be contaminated with *E. coli* (IRR: 0.2; CI: [0.0–0.6]; $p = 0.005$) and (IRR: 0.1; CI: [0.0–0.9]; $p = 0.039$), respectively. However, containers manually filled with rainwater were more likely to be contaminated with *E. coli* compared to those that were not (IRR: 8.9; CI: [2.9–26.7]; $p < 0.001$). Similarly in rural Thailand, containers manually filled with rainwater were also more likely to be contaminated with *E. coli* compared to those that were not (IRR: 11.9; CI: [5.5–25.9]; $p < 0.001$).

Among the household water management practices, the type of storage containers and mode of collecting water to drink were the only factors significantly associated with the occurrence of *E. coli* in DW, but only in Laos (Table 4). In suburban and rural Laos, buckets and jars, as storage containers, were less likely to be contaminated with *E. coli* than other kinds of containers. In suburban Laos, DW in households where members scooped water from containers was more likely to be contaminated with *E. coli* than those pouring the water out (IRR: 4.3; CI: [1.1–16.1]; $p = 0.033$). In rural Laos, DW in containers covered with lids were more likely to be contaminated with *E. coli* than those without lids (IRR: 9.2; CI: [2.4–35.7]; $p = 0.001$).

Water containers in households without a toilet facility were more likely to be contaminated with *E. coli* compared to households using pour flush toilet in Laos (Table 4). In suburban and rural Laos, households without a toilet facility were over 4 times (IRR: 4.8; CI: [1.3–18.1]; $p = 0.02$) and 14 times (IRR: 14.2; CI: [3.5–56.9]; $p < 0.001$) more likely to be contaminated with *E. coli* compared to households using pour flush toilets. In rural Laos, DW containers in households with a handwashing facility with soap near or inside the toilet were significantly less likely to be contaminated with *E. coli* (IRR: 0.2; CI: [0.0–0.6]; $p = 0.008$) compared to those without a handwashing facility with soap.

Table 4 | IRR ([95% confidence intervals] *p*-value) by univariate analysis of *E. coli* in stored DW (*E. coli* MPN/100 mL) related to socio-demographic, DW sources, household water management, sanitation and hygiene in suburban and rural villages in Laos and Thailand

		Laos		Thailand	
		Suburban	**Rural**	**Suburban**	**Rural**
No. of containers		139	145	178	268
Socio-demography					
Literacy	No	1	1	1	1
	Yes	2.2 [0.3–15.1] 0.423	0.3 [0.0–2.5] 0.244	0.7 [0.0–17.5] 0.815	na
Occupation group	Agriculture	1	1	1	1
	Commerce	na	0.03 [0.0–1.6] 0.086	2.6 [0.7–9.7] 0.165	0.1 [0.0–18.1] 0.435
	Service	Na	**0.01 [0.0–0.6] 0.025**	1.0 [0.1–16.9] 0.997	0.04 [0.0–2.4] 0.128
	Other[a]	na	na	2.1 [0.6–7.3] 0.225	**0.02 [0.0–0.8] 0.035**
Room occupancy rate	≤2.5 persons/room	1	1	1	1
	>2.5 persons/room	1.4 [0.4–5.7] 0.609	**0.1 [0.0–0.4] < 0.001**	0.8 [0.2–2.6] 0.696	0.4 [0.1–1.0] 0.062
Wealth status	Poor	1	1	1	1
	Intermediate	0.3 [0.1–1.1] 0.066	**0.2 [0.0–0.9] 0.045**	0.9 [0.0–58.3] 0.997	0.4 [0.1–1.4] 0.145
	Rich	**0.01 [0.0–0.1] < 0.001**	0.1 [0.0–2.2] 0.162	0.5 [0.0–30.5] 0.766	**0.1 [0.0–0.4] 0.001**
Housing material	Cement and wood	1	1	1	1
	Cement	na	na	1.5 [0.4–6.1] 0.531	**10.3 [2.7–40.3] 0.001**
	Wood	na	4.2 [0.8–22.2] 0.087	1.6 [0.3–7.5] 0.545	**9.0 [3.5–23.3] < 0.001**
Sources of DW (most used)					
Rain-fed	No	1	1	1	1
	Yes	2.4 [0.3–15.9] 0.38	**0.2 [0.0–0.6] 0.005**	1.7 [0.5–6.5] 0.417	na
Manually collected rain	No	1	1	1	1
	Yes	0.2 [0.0–1.2] 0.078	**8.9 [2.9–26.7] < 0.001**	0.6 [0.2–1.9] 0.406	**11.9 [5.5–25.9] < 0.001**
Purchased bottled water	No	1	1	1	1
	Yes	0.3 [0.1–1.1] 0.06	**0.1 [0.0–0.9] 0.039**	1.1 [0.4–2.9] 0.782	0.01 [0.0–2.6] 0.109
Borehole	No	1	1	1	1
	Yes	2.2 [0.4–12.9] 0.369	na	na	na
Household water management					
Types of DW storage containers					
Jar	No	1	1	1	1
	Yes	3.7 [0.7–19.5] 0.123	**0.2 [0.1–0.7] 0.012**	1.9 [0.5–7.6] 0.331	0.5 [0.2–1.2] 0.134
Bucket	No	1	1	1	1
	Yes	**0.1 [0.0–0.7] 0.014**	1.0 [0.3–3.9] 0.957	1.1 [0.4–2.9] 0.925	2.2 [0.9–5.8] 0.096
Bottle	No	1	1	1	1
	Yes	1.1 [0.3–4.6] 0.869	0.4 [0.1–1.6] 0.193	0.3 [0.0–2.2] 0.214	na
Lid status	without lid	1	1	1	1
	with lid	na	**9.2 [2.4–35.7] 0.001**	na	14.5 [0.4–474.2] 0.133
Mode of collecting water to drink	Pouring into cup	1	1	1	1
	Scooping into cup	**4.3 [1.1–16.1] 0.033**	1.6 [0.4–6.0] 0.453	0.7 [0.2–2.5] 0.607	0.7 [0.3–1.8] 0.47
Frequency of container cleaning	At least biweekly	1	1	1	1
	Monthly	na	0.01 [0.0–1.2] 0.058	1.2 [0.0–61.9] 0.926	8.7 [0.9–82.0] 0.058
	>Monthly to yearly	na	0.01 [0.0–15.3] 0.23	1.5 [0.4–5.9] 0.517	1.0 [0.4–2.4] 0.994

(*continued*)

Table 4 | continued

		Laos		Thailand	
		Suburban	**Rural**	**Suburban**	**Rural**
Sanitation facility	Pour flush toilet	1	1	1	1
	Flush toilet	0.1 [0.0–1.3] 0.075	0.5 [0.0–408.4] 0.82	1.4 [0.4–5.7] 0.624	na
	No toilet	**4.8 [1.3–18.1] 0.02**	**14.2 [3.5–56.9] < 0.001**	na	na
Hygiene					
Hand washing facility with soap near or inside the toilet	No	1	1	1	1
	Yes	0.9 [0.2–3.7] 0.901	**0.2 [0.0–0.6] 0.008**	1.6 [0.5–5.8] 0.46	na

Abbreviation: na, not applicable.
IRRs in bold are significant at $p \leq 0.05$.
[a]Retired, unemployed and student.

Multivariate analysis

As with the univariate analysis, results from the multivariate analysis revealed that the main factors contributing to the occurrence of *E. coli* in stored DW at the household level was country specific and varied from location to location (Table 5).

DW stored in wooden houses were more likely to be contaminated with *E. coli* in suburban Laos (IRR: 8.2; CI: [1.9–34.9]; $p = 0.004$) and rural Thailand (IRR: 2.7; CI [1.1–6.5]; $p = 0.031$), than DW stored in houses made from both cement and wood.

Rain-fed water in rural Laos and suburban Thailand, and manual filling of containers with rainwater in rural Thailand were significantly associated with the occurrence of *E. coli* in DW containers (Table 5). Specifically, in rural Thailand the occurrence of *E. coli* in stored DW was nearly 34 times more likely in households manually filling their containers with rainwater than in households that were not (IRR: 33.6; CI: [9.2–122.5]; $p < 0.001$). DW in rain-fed containers were less likely to be contaminated with *E. coli* than manually filled containers in rural Laos (IRR: 0.2; CI: [0.1–0.9]; $p = 0.044$), but the opposite was found in suburban Thailand (IRR: 33.5; CI: [1.4–771.5]; $p = 0.028$).

DW containers with lids were nearly six times more likely to be contaminated with *E. coli* than those without lids in rural Laos (IRR: 5.6; CI: [1.3–24.5]; $p = 0.023$). There was a higher occurrence of *E. coli* contamination in DW stored in jars compared with other storage containers in suburban Laos (IRR: 8.9; CI: [0.9–79.4]; $p = 0.05$) and suburban Thailand (IRR: 23.0; CI: [2.2–244.4]; $p = 0.009$). Also, storage of DW in buckets increased the occurrence of *E. coli* contamination in suburban Thailand (IRR: 4.9; CI: [1.1–22.5]; $p = 0.041$), whereas in suburban Laos, water stored in bottle were more likely to be contaminated with *E. coli* compared with non-bottled water (IRR: 20.7; CI: [4.0–106.0]; $p < 0.001$). In suburban Thailand, DW containers that were cleaned less frequently (monthly and longer, up to yearly), were less likely to be contaminated with *E. coli* compared with those that were cleaned more frequently (IRR: 0.01; CI: [0.0–0.6]; $p = 0.026$). However, in rural Thailand, containers that were less frequently cleaned (monthly and longer, up to yearly) were more likely to be contaminated with *E. coli* than containers that were cleaned more frequently (IRR: 7.9; CI: [2.2–28.5]; $p = 0.001$). The occurrence of *E. coli* in stored DW was around 7 times (IRR: 7.2; CI: [1.6–31.5]; $p = 0.009$) and nearly 17 times (IRR: 16.6; CI: [3.8–65.3]; $p < 0.001$) more likely in households without a toilet facility compared with households using pour flush toilet in suburban and rural Laos respectively.

DISCUSSION

The occurrence of *E. coli* in DW storage containers at the household level in Laos and Thailand was a function of several factors that varied from village to village. These factors resulted in high concentrations of *E. coli* (>1,000 *E. coli* MPN/100 mL) in stored DW across the study areas in

Table 5 | IRR ([95% confidence intervals] *p*-value) by multivariate analysis of *E. coli* in stored DW (*E. coli* MPN/100 mL) related to socio-demographic, DW sources, household water management and sanitation in suburban and rural villages in Laos and Thailand

		Laos		Thailand	
		Suburban	**Rural**	**Suburban**	**Rural**
No. of containers		139	145	178	268
Socio-demography					
Housing material	Cement and wood	1			1
	Cement	0.1 [0.0–1.3] 0.087			0.7 [0.1–3.8] 0.699
	Wood	**8.2 [1.9–34.9] 0.004**			**2.7 [1.1–6.5] 0.031**
Sources of DW					
Rain-fed	No		1	1	
	Yes		**0.2 [0.1–0.9] 0.044**	**33.5 [1.4–771.5] 0.028**	
Manually collected rain	No				1
	Yes				**33.6 [9.2–122.5] < 0.001**
Borehole	No	1			
	Yes	4.8 [0.9–25.9] 0.067			
Household water management					
Types of DW storage containers					
Jar	No	1		1	
	Yes	**8.9 [0.9–79.4] 0.05**		**23 [2.2–244.4] 0.009**	
Bucket	No			1	
	Yes			**4.9 [1.1–22.5] 0.041**	
Bottle	No	1			
	Yes	**20.7 [4.0–106.0] < 0.001**			
Lid status	Without lid		1		
	With lid		**5.6 [1.3–24.5] 0.023**		
Frequency of container cleaning	At least biweekly			1	1
	Monthly			**0.01 [0.0–0.5] 0.024**	8.6 [0.6–121.7] 0.112
	>Monthly to yearly			**0.01 [0.0–0.6] 0.026**	**7.9 [2.2–28.5] 0.001**
Sanitation facility	Pour flush toilet	1	1		
	Flush toilet	0.5 [0.0–4.9] 0.530	0.4 [0.0–249.5] 0.774		
	No toilet	**7.2 [1.6–31.5] 0.009**	**16.6 [3.8–65.3] < 0.001**		

IRRs in bold are significant at $p \leq 0.05$.

both countries. The exception to this was suburban Thailand; where the concentrations of *E. coli* in DW were lower (≤105 *E. coli* MPN/100 mL). Stored DW in containers in Laos had higher *E. coli* contamination levels than in Thailand. The relatively high *E. coli* concentrations in the two villages in Laos and in rural Thailand are a potential risk factor for gastroenteric diseases, particularly diarrhoea. Children in the study areas, especially those less than two years could potentially be at risk of diarrhoeal disease transmission as observed in an earlier study conducted in the Philippines (Moe *et al.* 1991).

Socio-demographic characteristics such as occupation of household heads, room occupancy rate, and wealth status were significantly associated with the occurrence of *E. coli* in stored DW only in the univariate analysis. Furthermore, housing material was significantly associated with *E. coli* in the multivariate analysis especially in suburban Laos and rural Thailand, where the mean *E. coli* concentrations in DW containers were higher if house construction material was wood (Table 3a and 3b). Wooden houses in all study villages were generally characterized by low wealth status. Low wealth status was significantly associated with an increased occurrence of *E. coli* in DW (Table 4) and therefore could have high occurrence of *E. coli* in their stored DW compared with houses constructed with both cement and wood (Table 5).

Rainwater, which is frequently used in all villages, was significantly associated with *E. coli* contamination in both the univariate and the multivariate analysis. DW containers with rain-fed water in rural Laos were significantly less likely to be contaminated with *E. coli* but this was not same for suburban Thailand where rain-fed water had a significantly higher level of *E. coli* contamination. In the study areas in Laos, rain-fed water was directly collected through connected pipes from rooftops. As a result, the chance of household members dipping their hands into water during collection is much lower compared with containers manually filled by hand. Furthermore, only small rain jar containers (200 mL) that are cleaned frequently on biweekly are used to store rainwater in rural Laos. Manually collected rainwater in rural Thailand also had a higher level of *E. coli* (Table 5). Rain-fed water in suburban Thailand and the manually collected rainwater in rural Thailand is unsafe to drink and therefore a considerable risk factor because of the increased occurrence of *E. coli* contamination as well as *E. coli* contamination levels not meeting the WHO standards (WHO 2006). In suburban Laos, although borehole water is less used compared to rainwater and purchased bottled water, it had the highest levels of *E. coli* contamination (Table 3a) and also a higher likelihood of contamination (IRR: 4.8; CI: [0.9–25.9]; $p = 0.067$) in the multivariate analysis compared to non-borehole water (Table 5).

Previous studies have shown that household water management play a role in *E. coli* contamination of stored DW (Jensen *et al.* 2002; Clasen & Andrew 2003). This was also confirmed in our current study where household water management practices were associated with *E. coli* contamination of stored DW (Table 5). In rural Laos, although nearly 100% of DW containers were said to be cleaned at least biweekly and 80% of them were covered with lids (Table 3a), the containers covered with lid had a significantly more occurrence of *E. coli* contamination (Tables 4 and 5). This is in contrast with findings made in previous studies (Chidavaenzi *et al.* 1998; Mazengia *et al.* 2002), where faecal and total coliform counts were 50% lower in containers covered with a lid compared to those without a lid. This may be explained by the fact that nearly 70% of DW in rural Laos came from the manually collected rainwater contaminated with high numbers of *E. coli* during the collection process (Table 3a). It has been shown that *E. coli* counts can increase with duration or time of storage (Jenkins *et al.* 2011). Also contamination of the water source could also explain this (Jensen *et al.* 2002; Wright *et al.* 2004). However, it should be stressed that as this was a cross-sectional study, the time-varying effect of *E. coli* levels in containers with and without lids was not accounted for. There is therefore a need for further studies accounting for the effect of water sources as well as duration of stored water on *E. coli* contamination in containers with and without lids.

In suburban and rural Thailand, the frequency of cleaning containers was significantly associated with the occurrence of *E. coli* in stored DW. In rural Thailand, less frequent cleaning of containers (once per month or less frequent) increased *E. coli* contamination of DW compared with biweekly cleaning (Table 5). The most commonly used DW containers in rural Thailand were rain jars (Table 3b), which are usually up to 2,000 liters (Dada *et al.* 2013). Containers of this size or capacity are not easy to clean; hence, water storage in such containers could lead to deterioration in water quality over time. In contrast to rural Thailand, containers that were less frequently cleaned (cleaned monthly and less often than monthly) in suburban Thailand were significantly less likely to be contaminated with *E. coli* (Table 5). A low room occupancy rate (Table 2) as well as access to other sources of DW such as bottled water in suburban Thailand might explain this (Table 3b). A low room occupancy rate combined with access to other sources of DW reduces household members' interaction/contact with stored DW thereby reducing the potential for contamination.

This study revealed that access to improved toilet facilities could provide a significant barrier against the contamination of stored DW within the household environment. Lack of access to toilet facilities was significantly associated with *E. coli* contamination of DW. This was particularly evident in Laos where a significant proportion of households in suburban and rural villages were without toilet facilities (Table 2). DW in households without toilets in suburban and rural Laos had high levels of *E. coli* contamination (Table 3a), and were significantly associated with the occurrence of *E. coli* in DW in the univariate and the multivariate analysis. Members of households without toilet facilities often resort to open defaecation without any handwashing facilities, and are therefore more likely to contaminate stored DW. Also the improper containment of human excreta resulting from the lack of toilet facilities can potentially provide other pathways for the contamination of stored DW within the household environment; and increase the risk of diarrhoeal disease transmission (Tumwine *et al.* 2002).

CONCLUSIONS

We conclude that the concentration of *E. coli* in stored DW in the household environment is higher in Laos than in Thailand, especially in households without toilets in both villages in Laos, as well as in the rural rather than suburban villages of both countries. Different factors contribute to the deterioration of water quality, and these vary across villages. From the final analysis, the factors that were significantly associated with the occurrence of *E. coli* in DW in suburban Laos were wooden house material, jars and bottles, and households without toilets; whereas in rural Laos the factors were rain-fed water, containers covered with lids and households without toilets. In suburban Thailand significant factors associated with the occurrence of *E. coli* in DW were rain-fed containers, jars, buckets and container cleaning frequency; whereas in rural Thailand they were wooden house material, manually collected rainwater and container cleaning frequency. This study revealed that socio-demographic characteristics were less associated with *E. coli* contamination in DW, compared to sanitation and hygiene. This may not always be the case in every setting, as all of these factors (Table 5) generally have been associated with *E. coli* hence, each or any combination of these factors serve as potential risk factors for faecal contamination. The levels of *E. coli* contamination found in all study sites were above the WHO drinking-water quality guidelines. Health education for appropriate treatment of stored DW (e.g. boiling) prior to drinking can provide a significant barrier against diarrhoeal disease incidence in households with poor water quality. In Laos, interventions related to the provision of improved toilet facilities have the potential of improving the quality of stored DW.

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PAPER II

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Parasites & Vectors

RESEARCH Open Access

Effects of socio-demographic characteristics and household water management on *Aedes aegypti* production in suburban and rural villages in Laos and Thailand

Nanthasane Vannavong[1,2*], Razak Seidu[3], Thor-Axel Stenström[4], Nsa Dada[1] and Hans J Overgaard[1,5]

Abstract

Background: Dengue fever is a mosquito-borne disease accounting for 50–100 million annual cases globally. Laos and Thailand are countries in south-east Asia where the disease is endemic in both urban and rural areas. Household water storage containers, which are favourable breeding sites for dengue mosquitoes, are common in these areas, due to intermittent or limited access to water supply. This study assessed the effect of household water management and socio-demographic risk factors on *Aedes aegypti* infestation of water storage containers.

Methods: A cross-sectional survey of 239 households in Laos (124 suburban and 115 rural), and 248 households in Thailand (127 suburban and 121 rural) was conducted. Entomological surveys alongside semi-structured interviews and observations were conducted to obtain information on *Ae. aegypti* infestation, socio-demographic factors and water management. Zero-inflated negative binomial regression models were used to assess risk factors associated with *Ae. aegypti* pupal infestation.

Results: Household water management rather than socio-demographic factors were more likely to be associated with the infestation of water containers with *Ae. aegypti* pupae. Factors that was significantly associated with *Ae. aegypti* infestation were tanks, less frequent cleaning of containers, containers without lids, and containers located outdoors or in toilets/bathrooms.

Conclusions: Associations between *Ae. aegypti* pupae infestation, household water management, and socio-demographic factors were found, with risk factors for *Ae. aegypti* infestation being specific to each study setting. Most of the containers did not have lids, larvicides, such as temephos was seldom used, and containers were not cleaned regularly; factors are facilitating dengue vector proliferation. It is recommended that, in Lao villages, health messages should promote proper use and maintenance of tightly fitted lids, and temephos in tanks, which were the most infested containers. Recommendations for Thailand are that small water containers should be cleaned weekly. Furthermore, in addition to health messages on dengue control provided to communities, attention should be paid to larval control for indoor containers in rural villages. Temephos or other immature control measures such as the use of pyriproxyfen, antilarval bacteria, or larvivorous fish should be used where temephos resistance is prevalent. Dengue control is not possible without additional adult mosquito control and community participation.

Keywords: *Aedes aegypti*, Dengue, Laos, Thailand, Water storage

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Background

About 2.5 billion people are globally at risk of dengue, and 50–100 million cases of dengue fever are reported each year [1], but the number of cases is likely to be much higher [2]. The transmission of this mosquito-borne disease is considered urban, but it also occurs in rural areas [3–8]. The disease is caused by four serotypes of the dengue virus and is transmitted by two main mosquito species, *Aedes aegypti* and *Ae. albopictus* [9], which are both vectors of chikungunya and Zika viruses as well. The Lao People's Democratic Republic (hereafter Laos) and Thailand are dengue-endemic, and all four dengue serotypes have been reported in both countries [10, 11]. Based on the national dengue surveillance data from 2006–2012 in Laos [11], one outbreak in 2010 was recorded resulting in 46 deaths. Several outbreaks have been reported in Thailand during 2000–2011, with the largest in 2010 resulting in 139 deaths [12]. A three-fold increase in the morbidity rate occurred in Laos between 2009–2010 (from 119 to 367 cases/100,000 people) while the corresponding figures for Thailand was a two-fold increase (89–184 cases/100,000 people). In the south of Laos, dengue is the most common cause of non-malaria fevers [13, 14].

Because of water scarcity, poor infrastructure and intermittent operation of water supply, the storage of water at the household level is common in many parts of the developing world, including Laos and Thailand [15, 16]. In both countries, water storage containers such as cement jars, tanks and others of various sizes have been used extensively for decades [17, 18]. Jars are normally used for storing drinking, and non-drinking water from rain and other sources piped to the house, while tanks are mostly used to store non-drinking water in toilets and bathrooms for bathing, laundry and cleaning [19]. However, as a result of improper household water management, these containers have become the preferred breeding sites for *Ae. aegypti* and an important risk factor for dengue fever transmission [17, 20–22].

Socio-demographic factors are known to affect dengue vector production and transmission. For instance, the risk of dengue in Thailand was associated with people gaining at least secondary education level and with households of more than four members [23]. Dengue modelling studies show that cases of dengue fever have a strong positive association with population density [24, 25]. Economic conditions were found to be associated with dengue cases, e.g. the seropositivity (immunoglobulin M, immunoglobulin G) of dengue was significantly associated with the absence of air-conditioning in households [26]. However, these socio-demographic factors may vary depending upon setting and other complexities of the communities like socio-economic dynamics, peoples' knowledge and behaviour, culture and geography.

Studies on dengue risk factors associated with household water storage, management and socio-demographic characteristics have rarely been conducted, particularly in Laos. According to a previous study conducted in southern Laos and north-eastern Thailand, high values of *Stegomyia* indices and *Ae. aegypti* production in water storage containers was identified [19]. Our study was conducted to identify the risk factors of household water management and socio-demographic characteristics on *Ae. aegypti* infestation in domestic water containers. Previously selected suburban and rural villages [19], one each in Laos and Thailand, were included in this study. Results from studies like this may provide important information for *Ae. aegypti* control programs to address the increasing threat of arboviral diseases, especially in light of the recent spread of Zika outbreaks.

Methods

Study areas

The study was conducted from the end of February to the beginning of June 2011, corresponding to the dry to the early wet season. One suburban and one rural village each in Thailand (Feb-April) and Laos (May-June) were surveyed. The selected villages in Laos were suburban Ban Lakhonesy (15°53'29.18"N, 105°33'56.59"E) and rural Ban Okadnavien (15°55'22.37"N, 105°31'35.0"E) in Salavan province, Southern Laos. In Thailand, the villages selected were suburban Ban Han (16°07'50.71"N, 102°32'5.81"E) and rural Ban Waileum (16°10'48.95"N, 102°28'15.61"E), Khon Kaen province, northeastern Thailand (Fig. 1). The villages were selected based on previously described criteria [19].

Study design

A cross-sectional survey of 248 households in Thailand (127 suburban and 121 rural), and 239 in Laos (124 suburban and 115 rural) was conducted. Entomological surveys alongside semi-structured interviews and observations were conducted to obtain information on *Ae. aegypti* infestation, socio-demographic factors and water management. In Thailand, the study sample represented 47 and 87% of all households in the selected suburban and rural villages, respectively. The corresponding numbers in Laos were 58% of the suburban households and 88% of the rural ones.

Household socio-demographic characteristics

Semi-structured interviews with the heads of each selected household (respondents) were conducted in the villages. Personal information of each respondent, such as age, sex, education level and occupation, were obtained. Education was categorised into two levels: primary school or less and more education than primary school.

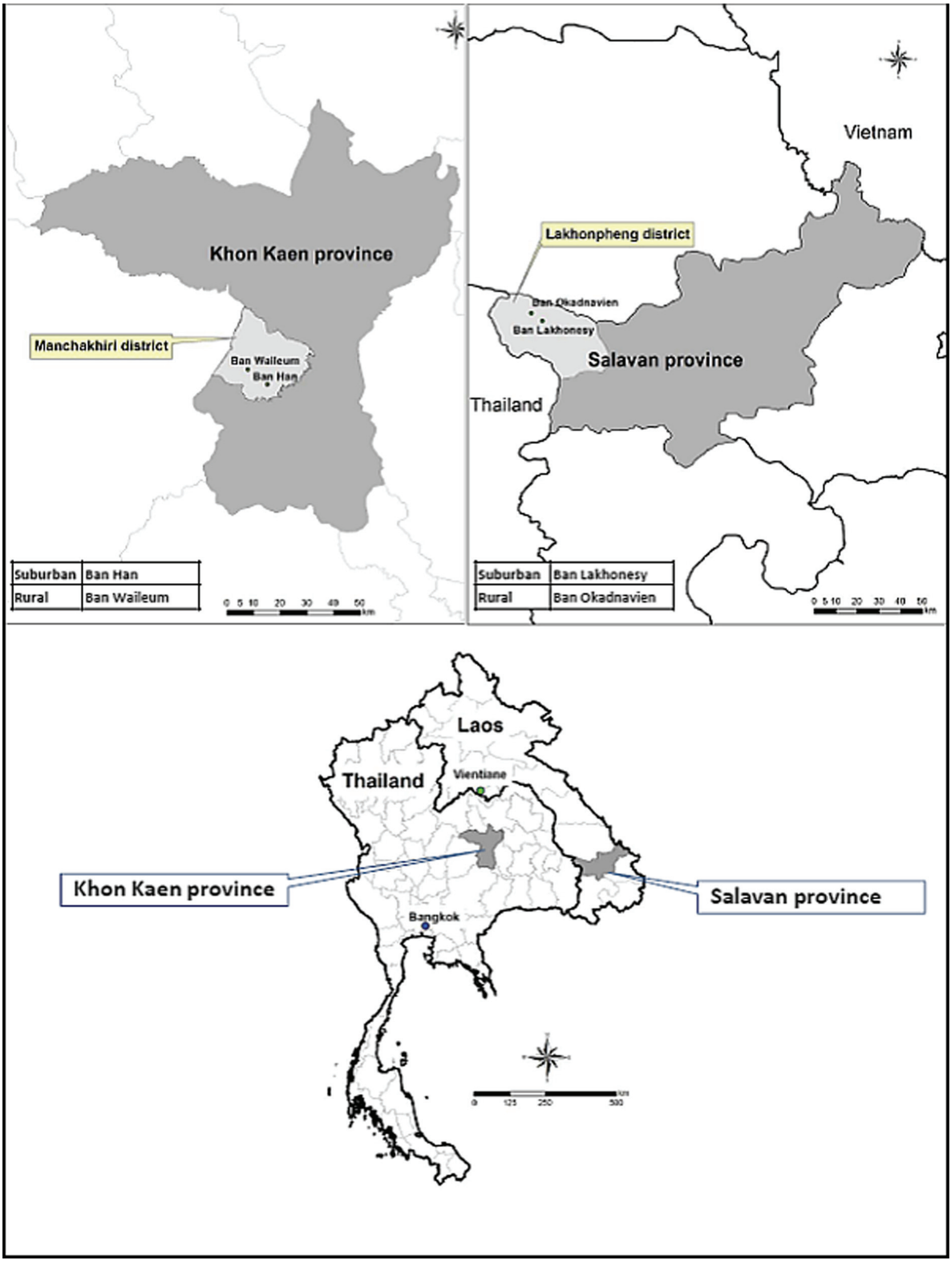

Fig. 1 Study villages in Laos and Thailand. Reprinted from Dada et al. (2013) Relationship between *Aedes aegypti* production and occurrence of *Escherichia coli* in domestic water storage containers in rural and sub-urban villages in Thailand and Laos. *Acta Tropica*, 126:177–185, with permission from Elsevier [19]

Occupation was categorised into agriculture, commercial (e.g. shopkeepers and other business), service, and others (retired, unemployed or student). The main occupation of the people in all study villages was agriculture, especially rice farming although some people in Thailand, but not in Laos, also grow sugarcane and cassava. Information related to households' ownership of durable assets, habitable room occupancy and access to water was also collected. In addition to the semi-structured interviews, observations were made of house material and recorded.

Household water management and entomological survey

As part of the household water management survey, all water storage containers were classified according to type, presence or absence of a lid, the frequency of refill, and location. The sources of the household water were characterized as rain-fed (rainwater that is collected directly from the rooftop, through the roof connected tube or from a metal roofing sheet), manually collected rainwater (rainwater collected manually from larger containers), piped water into the household, or borehole water (i.e. boreholes or protected drilled wells owned by households and located in the housing areas). Containers were defined as being indoors if located under the main roof of the house or outdoors if located outside the house or under the eaves of the house. Containers in bathrooms/toilet were classified as a separate group (i.e. neither indoors nor outdoors). All household containers used for water storage were examined for mosquito pupae and larvae. If present, pupae were collected, counted, and brought back to the field station for identification using a dissecting microscope and illustrated keys as previously described [19]. All *Aedes* pupae were identified to *Ae. aegypti* and *Ae. albopictus.* Only thirteen pupae from the Lao study villages (5 suburban and 8 rural) were identified as *Ae. albopictus.* Therefore, this species was excluded in the analysis. A number of pupae were used as a dependent variable in the model of zero-inflated negative binomial regression (ZINB).

Data analysis

Descriptive analysis of socio-demographic and household water management characteristics was conducted for each study village. Further analysis was undertaken to derive additional risk factors such as room occupancy rate and wealth status of the households. Estimation of the room occupancy rate was based on United Nation's definition [27]. Wealth status of the households was ranked into rich, intermediate and poor using Principal Components Analysis (PCA) based on group weighted mean scores [28]. The variables used in the wealth status ranking are presented in Table 1.

Table 1 Variables used in the wealth status ranking

Variables	Options/Values
House material	Cement/wooden/cement-wood
House floor material	Cement/wooden/cement-wood
Room occupancy rate	> 2.5 persons per habitable room/ ≤ 2.5 persons per habitable room
Ownership of durable assets	Mobile phone/cell phone/TV/radio/ refrigerator/car/motorcycle/bicycle
Affordability of bottled water	Can afford/cannot afford
Ownership of toilet facility	Yes/No
Ownership of flush toilet	Yes/No
Ownership of pour flush toilet	Yes/No

Univariate ZINB regression model was used to assess the independent effect of each of the socio-demographic and water management risk factors on the number of *Ae. aegypti* pupae in water containers. All factors included in the univariate analysis were then entered into a multivariate model to find the correlation between different factors and *Ae. aegypti* infestation in household storage water containers; and to eliminate confounding factors. The significant factors in the multivariate models were derived using backwards selection procedure. The unit of analysis with the ZINB model was the container. Statistical analyses were carried out using the statistical software SPSS 20.0 (IBM Corp.) and STATA (version 10; STATA Corporation, College Station, TX, USA). For the raw data used in the analyses please see Additional file 1.

Results

General information of study villages

The general description of the study villages is shown in Table 2. In both rural villages, the majority of the respondents were farmers (94.8 in Laos and 95.9% in Thailand). The level of education was low in both rural villages with 95.7 and 91.7% of the respondents having no more than primary education in Laos and Thailand, respectively. In both suburban villages, on the other hand, at least 47% of respondents had at least primary education. The room occupancy rate of > 2.5 persons/habitable room was lower in Thailand compared to Laos. In Thailand, 83 and 75% in the suburban and rural village respectively had a room occupancy rate of ≤ 2.5 persons/habitable room. In Laos, this room occupancy rate was 50% in the suburban village and 63% in the rural village (Table 2). The socio-economic status (SES) was higher in Thailand compared to Laos with 60 and 31% of the households in suburban and rural Thailand falling under the rich category, respectively.

Table 2 General information of respondents (household heads) and their households in a suburban and a rural village in Laos and Thailand (percentages in parentheses)

		Laos		Thailand	
		Suburban	Rural	Suburban	Rural
No. of households		124	115	127	121
Gender	Male	40 (32.3)	48 (41.7)	66 (51.9)	88 (72.7)
	Female	84 (67.7)	67 (58.3)	61 (48.1)	33 (27.3)
Occupation	Agriculture	66 (53.2)	109 (94.8)	30 (23.6)	116 (95.9)
	Commercial	12 (9.7)	3 (2.6)	34 (26.8)	1 (0.7)
	Service	30 (24.2)	3 (2.6)	11 (8.7)	2 (1.7)
	Other[a]	16 (12.9)	0	52 (40.9)	2 (1.7)
Education level	≤ Primary school	58 (46.8)	110 (95.7)	77 (60.6)	112 (92.6)
	> Primary school	66 (53.2)	5 (4.3)	50 (39.4)	9 (7.4)
Room occupancy rate	> 2.5 persons/room	62 (50.0)	43 (37.4)	22 (17.3)	30 (24.8)
	≤ 2.5 persons/room	62 (50.0)	72 (62.6)	105 (82.7)	91 (75.2)
Housing material	Cement and wood	48 (38.7)	16 (13.9)	88 (69.3)	74 (61.2)
	Cement	25 (20.2)	1 (0.9)	27 (21.3)	15 (12.4)
	Wood	51 (41.1)	98 (85.2)	12 (9.4)	32 (26.4)
Floor material	Cement and wood	29 (23.4)	14 (12.2)	82 (64.6)	62 (51.3)
	Cement	39 (31.5)	6 (5.2)	30 (23.6)	26 (21.5)
	Wood	53 (42.7)	95 (82.6)	14 (11.0)	32 (26.4)
	Ground	3 (2.4)	0	1 (0.8)	1 (0.8)

[a]Retired, unemployed and student

Rural Laos had the highest proportion of poor households, 81% (Fig. 2).

Aedes aegypti pupae positive containers were found in all four study villages (Tables 3 and 4). In Thailand, 57 and 47% of the containers were positive for pupae in the suburban and rural village, respectively. In Laos, 54% in the suburban and 33% in the rural village were pupae positive. The most important risk factors for *Ae. aegypti* pupal presence and abundance were container type (jars and tanks), location (toilets/bathrooms), lid status (no lids), education level (primary level or less, except for suburban Laos), and SES (intermediate and rich households, except in rural Laos where 81% of the households were poor). However, some factors such as water source and container cleaning frequency were site specific.

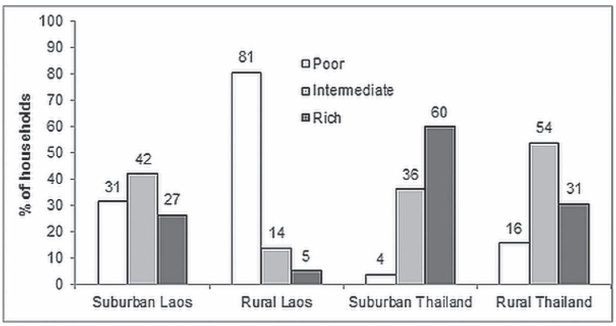

Fig. 2 The proportion of households classified as poor, intermediate, and rich in a suburban and a rural village in Laos and Thailand. Numbers above each bar represent the percentage of households within each location

Table 3 Number of containers positive for *Ae. aegypti* out of those inspected and total number and density of pupae collected in relation to socio-demographic and household water management characteristics in Laos

		Suburban village				Rural village			
		Containers		Pupae		Containers		Pupae	
		No. positive/inspected	% positive	Total	No. per container (95% CI)	No. positive/inspected	% positive	Total	No. per container (95% CI)
Overall per study location		75/139	54.0	911	6.6 (4.5–8.6)	21/64	32.8	558	8.7 (2.0–15.4)
Socio-demography									
Education level	≤ Primary school	34/64	53.1	367	5.7 (3.3–8.1)	21/63	33.3	558	8.9 (2.1–15.6)
	> Primary school	41/75	54.7	544	7.2 (3.9–10.6)	0/1	0	0	–
Occupation	Agriculture	44/82	53.7	454	5.5 (3.6–7.5)	21/64	32.8	558	8.7 (2.0–15.4)
	Commercial	8/11	72.7	216	19.6 (0.3–39.0)	0	0	0	–
	Service	11/25	44.0	139	5.6 (0.9–10.2)	0	0	0	–
	Other[a]	12/21	57.1	102	4.9 (0.7–9.1)	0	0	0	–
Wealth status	Poor	21/41	51.2	240	5.9 (2.6–9.1)	19/54	35.2	514	9.5 (1.7–17.4)
	Intermediate	35/68	51.5	335	4.9 (2.8–7.1)	2/9	22.2	44	4.9 (-2.7–12.5)
	Rich	19/30	63.3	336	11.2 (3.9–18.5)	0/1	0	0	–
Household water management									
Container type	Jar	35/77	45.5	418	5.4 (2.6–8.2)	17/49	34.7	234	4.8 (1.8–7.8)
	Tank	29/41	70.7	389	9.5 (4.9–14.0)	4/6	66.7	324	54.0 (-21.6–129.6)
	Bucket	3/10	33.3	19	1.9 (-1.7–5.5)	0/7	0	0	–
Container location	Indoor	12/15	80.0	142	9.5 (3.5–15.4)	5/26	19.2	36	1.4 (-0.2–2.9)
	Outdoor	32/79	40.5	346	4.4 (1.9–6.9)	12/29	41.4	198	6.8 (1.9–11.7)
	Toilet/bathroom	31/45	68.9	423	9.4 (5.0–13.8)	4/9	44.4	324	36.0 (-12.5–84.5)
Household water source	Rain-fed	12/37	32.4	114	3.1 (0.7–5.5)	13/43	30.2	185	4.3 (0.9–7.6)
	Manually collected rain	3/3	100.0	14	4.7 (-2.9–12.3)	0/4	0	0	–
	Piped water	0	0	0	–	0	0	0	–
	Borehole	59/98	60.2	781	7.9 (5.2–10.8)	8/17	47.1	373	21.9 (-2.5–46.3)
Frequency of container cleaning	≤ Weekly	52/96	54.2	668	6.9 (4.2–9.8)	18/59	30.5	495	8.4 (1.2–15.5)
	> Weekly-monthly	22/42	52.4	238	5.7 (2.9–8.4)	3/4	75.0	63	15.8 (-12.7–44.2)
	> Monthly-yearly	1/1	100.0	5	5	0/1	0	0	–
Lid status	With lid	9/32	28.1	56	1.8 (0.0–3.5)	3/16	18.8	31	1.9 (-1.6–5.5)
	Without lid	66/107	61.7	855	7.9 (5.4–10.6)	18/48	37.5	527	10.9 (2.2–19.8)
Temephos present in container	No	53/108	49.1	668	6.2 (3.8–8.6)	19/60	31.7	446	7.4 (0.8–14.0)
	Yes	22/31	70.9	243	7.8 (3.2–12.4)	2/4	50.0	112	28.0 (-37.3–93.3)

Abbreviation: *CI* confidence interval
[a]Retired, unemployed and student

Table 4 Number of containers positive for *Ae. aegypti* out of those inspected and total number and density of pupae collected in relation to socio-demographic and household water management characteristics in Thailand

		Suburban village				Rural village			
		Containers		Pupae		Containers		Pupae	
		No. positive/inspected	% positive	Total	No. per container (95% CI)	No. positive/inspected	% positive	Total	No. per container (95% CI)
Overall per study location		97/171	56.7	1,046	6.1 (3.2–9.0)	85/179	47.5	1,007	5.6 (2.9–8.3)
Socio-demography									
Education level	≤ Primary school	71/115	61.7	833	7.2 (3.2–11.3)	72/161	44.7	658	4.1 (2.0–6.1)
	> Primary school	26/56	46.4	213	3.8 (1.1–6.6)	13/18	72.2	349	19.4 (-0.4–39.2)
Occupation	Agriculture	21/35	60.0	115	3.3 (1.3–5.3)	79/168	47.0	962	5.7 (2.9–8.6)
	Commercial	23/34	67.6	229	6.7 (1.4–12.1)	2/3	66.7	4	1.3 (-1.5–4.2)
	Service	5/13	38.5	8	0.6 (0.1–1.2)	2/3	66.7	13	4.3 (-8.4–17.1)
	Other[a]	48/89	53.9	694	7.8 (2.7–12.9)	2/5	40.0	28	5.6 (-7.9–19.2)
Wealth status	Poor	4/8	50.0	47	5.9 (-5.1–16.9)	8/19	42.1	30	1.6 (0.4–2.8)
	Intermediate	35/64	54.7	574	8.9 (1.9–15.9)	39/101	38.6	534	5.3 (1.7–8.9)
	Rich	58/99	58.6	425	4.3 (2.2–6.3)	38/59	64.4	443	7.5 (2.1–12.9)
Household water management									
Container type	Jar	38/69	55.1	370	5.4 (0.5–10.2)	41/100	41.0	429	4.3 (0.7–7.8)
	Tank	51/85	60.0	595	7.0 (2.9–11.1)	42/74	56.8	572	7.7 (3.3–12.1)
	Bucket	8/17	47.1	81	4.8 (-3.4–12.9)	2/5	40.0	6	1.2 (-0.8–3.2)
Container location	Indoor	24/44	54.5	106	2.4 (1.2–3.6)	18/56	32.1	335	5.9 (-0.3–12.3)
	Outdoor	11/20	55.0	247	12.4 (-4.8–29.5)	15/35	42.9	91	2.6 (0.6–4.6)
	Toilet/bathroom	62/107	57.9	693	6.5 (3.0–9.9)	52/88	59.1	581	6.6 (2.9–10.3)
Household water source	Rain-fed	8/15	53.3	15	1.0 (0.1–1.9)	4/12	33.3	37	3.1 (-0.6–6.7)
	Manually collected rain	2/6	33.3	2	0.3 (-0.2–0.9)	9/34	26.5	120	3.5 (-1.9–8.9)
	Piped water	84/145	57.9	1,022	7.1 (3.7–10.4)	70/131	53.4	848	6.5 (3.1–9.9)
	Borehole	0	0	0	–	0	0	0	–
Frequency of container cleaning	≤ Weekly	37/71	52.1	168	2.4 (1.3–3.5)	27/60	45.0	146	2.4 (1.2–3.7)
	> Weekly-monthly	55/92	59.8	864	9.4 (4.1–14.6)	55/111	49.5	828	7.5 (3.2–11.7)
	> Monthly-yearly	5/8	62.5	14	1.8 (-0.2–3.7)	3/8	37.5	33	4.1 (-2.5–10.8)
Lid status	With lid	13/29	44.8	24	0.8 (0.2–1.5)	12/45	26.7	145	3.2 (-0.9–7.3)
	Without lid	84/142	59.2	1,022	7.2 (3.7–10.6)	73/134	54.5	862	6.4 (3.1–9.7)
Temephos present in container	No	97/171	56.7	1,046	6.1 (3.2–9.0)	83/177	46.9	983	5.5 (2.9–8.3)
	Yes	0	0	0	–	2/2	100.0	24	12.0 (-51.5–75.5)

Abbreviation: CI confidence interval
[a]Retired, unemployed and student

Effect of socio-demographic characteristics on *Aedes aegypti* production

The univariate analysis (Table 5) showed that households in suburban Laos where the respondent's occupation was 'commercial' were significantly associated with *Ae. aegypti* pupae abundance (IRR 2.9, 95% CI: 1.01–8.8) compared to agricultural households. In suburban Thailand, respondents involved in 'other' occupations (retired, unemployed or student), were about three times more likely to have *Ae. aegypti* pupae in their homes, whereas, those who were services were less likely to have *Ae. aegypti* in their homes (IRR 0.2, 95% CI: 0.1–0.8) compared to farmers' households. In the multivariate analysis, only 'other' occupations (IRR 2.3, 95% CI: 1.1–4.8) in suburban Thailand remained significantly associated with *Ae. aegypti* (Table 6). In rural Thailand, 'commercial' occupations were less likely to have *Ae. aegypti* infestation in their homes (IRR 0.1, 95% CI: 0.01–0.8) compared to those with agriculture occupation (Table 6). In rural Thailand, the houses of respondents who had a higher education than primary school were four times more likely to be infested with *Ae. aegypti* than in houses of respondents with lower education (Table 5). Households in rural Thailand assessed as being intermediate or rich were each about five times more likely to have their homes infested with *Ae. aegypti* compared to poor households (Table 5). In the multivariate model, these relationships became much stronger with households in the intermediate (IRR 9.3, 95% CI: 3.1–28.1) and rich SES categories (IRR 13.2, 95% CI: 4.01–43.3) being significantly associated with *Ae. aegypti* (Table 6).

Effect of household water management on *Aedes aegypti* production

Container types and locations

Jars and tanks were the most commonly used water storage containers across all four villages (Tables 3 and 4). The univariate model showed that container type was only significantly associated with *Ae. aegypti* pupae in rural Laos, and not in any other study village. Here, jars were the least likely to be infested (IRR 0.2, 95% CI: 0.1–0.5) when compared to non-jar containers, and tanks were the most likely to be infested (IRR 6.3, 95% CI: 2.0–19.9) when compared to non-tanks (Table 5). In the multivariate analysis, tanks remained the most likely to be infested (IRR 5.9, 95% CI: 1.9–19.1), while jars were not significant (Table 6).

In Laos, 57% of water storage containers in the suburban village and 45% in the rural village were located outdoors. In Thailand, 63% of the containers in the suburban village and 49% in the rural village were found in toilets or bathrooms (Tables 3 and 4). For rural Laos, the univariate model showed that containers located in the toilet/bathroom were about 13 times more likely to be infested with *Ae. aegypti* pupae than those located indoors (Table 5). Container location was not of importance in suburban Laos. In suburban Thailand, containers located in the toilet or bathroom (IRR 2.7, 95% CI: 1.3–5.6) and those located outdoors (IRR 5.9, 95% CI: 1.8–19.9) were more likely to be infested than indoor containers (Table 5). However, in rural Thailand, the opposite was observed; containers located outdoors were less likely to be infested compared to those located indoors (IRR 0.2, 95% CI: 0.1–0.8) (Table 5). In the multivariate model for rural Thailand, containers located outdoors (IRR 0.2, 95% CI: 0.1–0.5) and in the toilet/bathroom (IRR 0.4, 95% CI: 0.2–0.9) were significant less likely to be associated with *Ae. aegypti* pupae infestation (Table 6).

Water sources

In rural Laos, the univariate analysis showed that containers with water from boreholes and rainwater were significantly associated with *Ae. aegypti*. Containers with borehole water were 3.6 times (IRR 3.6, 95% CI: 1.2–11.1) more likely to be infested than containers with non-borehole water. Rain-fed water was significantly less likely to be infested with *Ae. aegypti* pupae than containers with other water sources (IRR 0.3, 95% CI: 0.1–0.9). Similar outcomes were obtained in suburban Thailand, with manually collected rainwater being less likely to be infested (IRR 0.1, 95% CI: 0.01–0.5) when other water sources were used as a reference. None of the water sources recorded was significantly associated with *Ae. aegypti* pupae in suburban Laos and rural Thailand. The multivariate model did not show any significant associations between water source and *Ae. aegypti* pupae across all four villages.

Frequency of container cleaning, lid status and a presence of temephos in container

In Laos, most of the containers in both rural (92%) and suburban (69%) villages were cleaned every week. The frequency of cleaning was not significantly associated with *Ae. aegypti*. In Thailand, the majority of the containers were cleaned less often than those in Laos. Fifty-four percent and 62% of the containers in suburban and rural Thailand, respectively, were cleaned once during a period of a week and up to one month (Tables 3 and 4). As a result, these containers were more likely to be associated with *Ae. aegypti* in both the suburban (IRR 4.2, 95% CI: 2.1–8.2) and the rural (IRR 3.5, 95% CI: 1.6–7.4) villages (univariate model) compared to containers that were cleaned once a week (Table 5). This association remained significant in the multivariate model in the suburban (IRR 3.5, 95% CI: 1.9–6.6) and the rural (IRR 2.6, 95% CI: 1.3–5.1) village, respectively (Table 6).

In all study villages, most of the containers did not have lids. Only 23 and 25% of those in suburban and rural Laos, and 17 and 25% in suburban and rural Thailand, respectively were covered. Containers with lids

Table 5 Incidence rate ratios, IRR (95% confidence intervals) by univariate analysis of *Ae. aegypti* pupae per container in relation to socio-demographic and household water management in a suburban and a rural village in Laos and Thailand

		Laos				Thailand			
		Suburban		Rural		Suburban		Rural	
No. of containers		139		64		171		179	
Socio-demography									
Education level	≤ Primary school	64	1	63	1	115	1	161	1
	> Primary school	75	1.3 (0.6–2.6)	1	na	56	0.6 (0.3–1.2)	18	4.1 (1.4–12.2)**
Occupation	Agriculture	82	1	64	1	35	1	168	1
	Commercial	11	2.9 (1.0–8.8)*	0	na	34	2.1 (0.8–5.1)	3	na
	Service	25	1.3 (0.5–3.3)	0	na	13	0.2 (0.1–0.8)*	3	na
	Other[a]	21	0.8 (0.3–1.9)	0	na	89	2.8 (1.2–6.3)*	5	na
Wealth status	Poor	41	1	54	1	8	1	19	1
	Intermediate	68	0.8 (0.4–1.8)	9	na	64	1.5 (0.3–8.6)	101	5.2 (1.8–15.2)**
	Rich	30	1.7 (0.7–4.2)	1	na	99	0.6 (0.1–3.5)	59	4.8 (1.7–13.6)**
Household water management									
Container type and location									
Jar	No	62	1	15	1	102	1	79	1
	Yes	77	0.9 (0.5–1.9)	49	0.2 (0.1–0.5)**	69	0.8 (0.4–1.5)	100	0.7 (0.3–1.5)
Tank	No	98	1	58	1	86	1	105	1
	Yes	41	1.2 (0.6–2.5)	6	6.3 (2.0–19.9)**	85	1.3 (0.7–2.5)	74	1.5 (0.7–3.1)
Bucket	No	129	1	57	1	154	1	174	1
	Yes	10	0.4 (0.1–2.5)	7	na	17	0.8 (0.2–2.9)	5	0.2 (0.0–1.9)
Container location	Indoor	15	1	26	1	44	1	56	1
	Outdoor	79	0.8 (0.3–2.1)	29	2.5 (0.8–7.4)	20	5.9 (1.8–19.9)**	35	0.2 (0.1–0.8)*
	Toilet/bathroom	45	1.1 (0.4–2.8)	9	12.8 (3.4–48.6)**	107	2.7 (1.3–5.6)**	88	0.6 (0.2–1.4)
Water source, cleaning and lids									
Rain-fed	No	102	1	21	1	156	1	167	1
	Yes	37	0.7 (0.3–1.8)	43	0.3 (0.1–0.9)*	15	na	12	0.7 (0.1–4.3)
Manually collected rain	No	136	1	60	1	165	1	145	1
	Yes	3	0.5 (0.1–3.1)	4	na	6	0.1 (0.0–0.5)**	34	1.2 (0.3–3.9)
Piped water	No	0		0		26	1	48	1
	Yes	0		0		145	na	131	1.2 (0.5–3.2)
Borehole	No	41	1	47	1	0		0	
	Yes	98	1.8 (0.8–4.2)	17	3.6 (1.2–11.1)*	0		0	
Frequency of container cleaning	≤ Weekly	96	1	59	1	71	1	60	1
	> Weekly-monthly	42	0.8 (0.4–1.7)	4	na	92	4.2 (2.1–8.2)**	111	3.5 (1.6–7.4)**
	> Monthly-yearly	1	0.5 (0.0–14.7)	1	na	8	0.7 (0.2–3.3)	8	2.4 (0.3–18.6)
Lid status	Without lid	107	1	48	1	142	1	134	1
	With lid	32	0.4 (0.1–1.1)	16	0.3 (0.1–1.8)	29	0.1 (0.0–0.3)**	45	0.9 (0.3–2.9)
Temephos present in container	No	108	1	60	1	171	1	177	1
	Yes	3	0.9 (0.4–1.8)	4	2.6 (0.3–21.5)	0	na	2	2.2 (0.1–56.2)

*$P \le 0.05$, **$P \le 0.01$

[a]Retired, unemployed and student

Abbreviation: *na* not applicable

Table 6 Incidence rate ratios, IRR (95% confidence intervals) by multivariate analysis of *Ae. aegypti* pupae per container in relation to socio-demographic and household water management in a suburban and a rural village in Laos and Thailand

		Laos				Thailand			
		Suburban		Rural		Suburban		Rural	
No. of containers		139		64		171		179	
Socio-demography									
Occupation	Agriculture					35	1	168	1
	Commercial					34	1.9 (0.8–4.9)	3	0.1 (0.0–0.8)*
	Service					13	0.6 (0.1–3.2)	3	0.9 (0.1–12.5)
	Other[a]					89	2.3 (1.1–4.8)*	5	1.5 (0.2–9.3)
Wealth status	Poor							19	1
	Intermediate							101	9.3 (3.1–28.1)**
	Rich							59	13.2 (4.0–43.3)**
Household water management									
Tank	No			58	1				
	Yes			6	5.9 (1.9–19.1)**				
Container location	Indoor	15	1					56	1
	Outdoor	79	0.6 (0.2–1.6)					35	0.2 (0.1–0.5)**
	Toilet/bathroom	45	0.7 (0.2–2.1)					88	0.4 (0.2–0.9)*
Frequency of container cleaning	≤ Weekly					71	1	60	1
	> Weekly-monthly					92	3.5 (1.9–6.6)**	111	2.6 (1.3–5.1)**
	> Monthly-yearly					8	2.4 (0.5–12.2)	8	5.9 (0.6-55.2)
Lid status	Without lid	107	1	48		142	1		
	With lid	32	0.3 (0.1–0.9)*	16	0.7 (0.2–2.6)	29	0.1 (0.0–0.4)**		

*$P \leq 0.05$, **$P \leq 0.01$
[a]Retired, unemployed and student

were significantly less likely to be infested than those without lids in the suburban villages in Laos (IRR 0.3, 95% CI: 0.1–0.9) and Thailand (IRR 0.1, 95% CI: 0.04–0.4) (Table 6). None of these associations was significant in the rural villages in either country (Tables 5 and 6).

In Laos, 22% of water storage containers in the suburban village and 6% in the rural village used the larvicide temephos (Abate). In Thailand, only one percent of containers in the rural village had temephos (Tables 3 and 4). There were no significant associations between temephos and *Ae. aegypti* pupae in both the univariate and multivariate models (Tables 5 and 6).

Discussion

The relationships between mosquito breeding and socio-economic and water management factors are complex as shown in the study. Several risk factors associated with *Ae. aegypti* pupae infestation were relatively site specific. In Thailand, both socio-demographic and water management factors were to different degrees related to immature *Ae. aegypti* production. Very few significant associations between immature *Ae. aegypti* production and socio-demographic and water management factors were found in suburban Laos. The rural village is excluded from comparisons since it was a homogenous poor low-educational agricultural community.

Socio-demographic relationships

Specifically, the occupation of the household head and household wealth status were significantly associated with *Ae. aegypti* infestation in Thailand. The significance of occupation varied and was site specific. In suburban Laos where the respondent's occupation was 'commercial', there were significant associations with *Ae. aegypti* pupae abundance (IRR 2.9, 95% CI: 1.01–8.8) compared to agricultural households (which was the reference). In suburban Thailand, significant associations were also found with *Ae. aegypti* infestation, but in households where the occupation of the household head was 'other' (retired, unemployed or student). Households with 'other' occupations are not economically active and were the largest group (41%) in the suburban village. Another study also showed that non-economically active people were about 1.6 times more likely to have their households present with *Ae. aegypti* [29]. Those with agricultural occupations had a lower likelihood in both suburban sites.

With regards to household wealth status in the rural Thai village, the intermediate households were nine times, and the rich households 13 times, more likely to have their home water containers infested with *Ae. aegypti* compared to the poorer households (Table 6). This may be because the intermediate and rich households had more water containers than the poor ones, thereby providing more breeding sites for *Ae. aegypti*. This contrasts a previous study in Colombian towns where water containers in rich households were less likely to be infested with *Ae. aegypti* immatures compared to poor households [30].

Water management factors

Cement tanks were significantly more likely to be infested with *Ae. aegypti* compared to other containers in Laos (Tables 5 and 6). In both the suburban and the rural villages, cement tanks without lids were used to store non-drinking water in the toilets or bathrooms. The major challenge for *Ae. aegypti* larval control in many countries in south-east Asia is that such tanks, which are difficult to cover, are more likely to be used on a large scale [19, 21, 31]. In addition, large containers are often difficult to clean more frequently to enable the interruption and prevention of mosquito life-cycle and mosquito production. Less frequent cleaning provides good breeding sites for dengue vector production [32–36]. It is thus not the container type as such that is a factor for consideration but rather the combination of container size, their placement, no or poorly fitted lids and low frequency of cleaning that is the combined determinant of *Aedes* infestation. This is further supported by the other types of containers, such as jars in Laos, which were significantly less likely to be infested with *Ae. aegypti,* especially in the rural village (Table 5) possibly due to their predominant use for the storage of drinking water and hence better handling (e.g. use of lids) and hygiene. This finding was contrary to those made in other studies where jars were considered a high-risk factor for mosquito breeding in Laos [37] and in Thailand [22], but again the combined purpose and handling practices will play a major role.

In the suburban village in Thailand, containers located outdoors and in toilets or bathrooms were more likely to be infested with *Ae. aegypti* than those located indoors, but in the rural village, outdoor containers were less likely to be infested (Table 5). It is unclear why this is so, but this could be because indoor containers in the suburban households were better handled and more often had lids than in the rural households. Rural households may provide better access for mosquitoes to indoor containers, which would be located in dark spaces, not well protected with lids and potentially providing attractive breeding sites. Again it is not only partly attributed to handling practices but also the purpose of the containers (example for drinking where the handling care is higher), our data showed there were more drinking water containers located outdoors in rural than in suburban villages of Thailand. In the suburban village outdoor containers were less often used for drinking (i.e. poorer hygiene). Other studies have shown that containers located outdoors are the main dengue vector producers compared to those located indoors [32, 34, 35, 38]. However, in Vietnam, the majority of *Ae. aegypti* immatures was found in indoor containers rather than outdoor containers [31, 39]. Our results show the complexity of *Ae. aegypti* breeding, as they breed in a wide range of *household containers* regardless of location, especially under similar environmental conditions.

The handling practices are further supported by the cleaning practises and container type. Containers in Thailand were less frequently cleaned than those in Laos, due to a higher frequency of large containers in Thailand (e.g. large cement jar containing up to 2,000 l of water). Containers cleaned on a weekly basis were less likely to be infested with *Ae. aegypti*. A weekly cleaning schedule is also recommended by many national public health authorities and by WHO [40, 41]. The effectiveness of frequent cleaning has also been confirmed by studies in northern Thailand [33] and in six other Asian countries [35].

This study also showed that containers with lids act as prevention against mosquito breeding. Containers with lids were significantly less likely to be infested with *Ae. aegypti* compared to those without lids in Laos (Table 6). This has also been shown in many other studies where containers without lids or partly covered produced more *Ae. aegypti* than those with lids [16, 31, 37, 42]. Container lids are not an absolute barrier and must be tightly fitted to prevent gravid females to enter for oviposition [43, 44]. Such lids are a low-cost, effective and environmental friendly intervention for dengue vector control and have also been recommended by the WHO [40]. However, this intervention needs to be properly managed and maintained by communities.

In rural Laos, containers with borehole water were almost four times more likely to be infested with *Ae. aegypti* pupae compared to containers with other water sources (Table 5). Many containers with borehole water (53%) (data not presented in results) were located in toilets or bathrooms, and all of them were without lids, all conditions that provide good breeding sites for *Ae. aegypti* [21, 31]. Conversely, rain-fed water was less likely to be infested compared to other water sources (Table 5). This could be because as many as 75% (data not presented in results) of the rain-fed containers were covered with lids and used for drinking. However, in the multivariate analysis

(Table 6), water sources were not significantly associated with *Ae. aegypti* infestation.

Laos and Thailand have similar dengue outbreak responses. The so-called Surveillance and Rapid Response Teams (SRRT) act rapidly, within 24 h when a dengue case is diagnosed by a physician, to implement vector control measures. Such measures usually consist of space spraying with thermal fog within a radius of 100 m from the affected house. In addition, the larvicide temephos (Abate® 1% sand granules) is freely provided nationwide in both countries. A systematic literature review showed that temephos was effective against *Ae. aegypti* production in water storage containers [45]. In the present study, it was found that some water containers in both villages of Laos contained temephos, but was mainly absent in Thailand (Tables 3 and 4). Thus, temephos was inconsistently used and may not have been an effective dengue control intervention in these settings. This could be due to problems of distribution or perceptions of temephos as a harmful chemical as well as improper use, which has been described in a study conducted in northeastern Thailand [46]. The indiscriminate use of temephos can lead to temephos resistance as identified in some parts of Thailand [47, 48]. Pyriproxyfen, spinosad, or antilarval bacteria (e.g. *Bti*) have been shown to be effective against *Ae. aegypti* [49] and could be used instead of temephos. Control measures such as the use of larvivorous fishes were not observed in the examined containers but were sometimes observed in containers in other households not included in the study. Personal protection using repellents to control adult mosquitoes was also observed, but not accounted for in this study. However, for any of these control measures to be effective against *Ae. aegypti*, the involvement of multi-sectoral stakeholders as well as active community participation is key [50].

This study was a cross-sectional survey carried out at the end of the dry and the beginning of the wet season. Risk factors related to household water management may vary between seasons, and between years. The number of rainwater storage containers in the wet season would be higher than observed in our study, providing more breeding sites. Also, our study might have influenced nearby households to take action to clean out positive mosquito containers in their homes and thus biasing the results.

Conclusions

This study showed a relationship between *Ae. aegypti* production in water storage containers and risk factors associated with socio-demography and households' water management practices. Most of the risk factors were specific to the study villages. Our study showed that household water management rather than socio-demographic factors were more likely to be associated with the infestation of water containers with *Ae. aegypti*. Most of the containers did not have lids, were not protected with larvicides and were not cleaned regularly, thereby providing breeding sites for dengue vectors. As the aforementioned risk factors were significantly associated with *Ae. aegypti* infestation, it is recommended that, in Lao villages, health messages should promote proper use and maintenance of tightly fitted lids, and temephos in tanks, which were the most infested containers. Recommendations for Thailand are that small water containers should be cleaned weekly. Furthermore, attention should be paid to larval control for indoor containers in a rural village in addition to health messages. Temephos, which is the first larval control method of choice today, can be used in areas without temephos resistance. Alternatively, pyriproxyfen, spinosad, antilarval bacteria (e.g. *Bti*) or larvivorous fish should be considered where temephos resistance is prevalent. However, adult mosquito control must also be considered in an integrated vector management strategy. Compliance is always an issue when it comes to mosquito control. Therefore, community participation will be key to the success of any selected control measure.

Abbreviations

IRR: Incidence rate ratios; PCA: Principal Components Analysis; SES: Socioeconomic status; SRRT: Surveillance and Rapid Response Teams; UN: United Nations; WHO: World Health Organization; ZINB: Zero-inflated negative binomial

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Availability of data and materials

The data supporting the conclusions of this article are included within the article. Raw data are provided in Additional file 1.

Authors' contributions

NV, HJO, TAS conceived and designed the study. NV conducted the field work, analysed the data and drafted the manuscript. RS contributed in the study design, supervised data and water sample collection and data analyses. ND conducted the entomological survey. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (No. 03/NECHR) on 17 December 2010 and by the Ethical Committee of Phramongkutklao College of Medicine, Bangkok, Thailand (S033h/53) on 21 March 2011. Informed signed consent was obtained from the household head of all participating households.

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PAPER III

Nanthasane Vannavong , Hans J Overgaard, Thor Axel Stenström, Razak Seidu. Assessing the risk factors of diarrhoeal disease in suburban and rural villages in Laos and Thailand. (Manuscript).

Assessing the risk factors of diarrhoeal disease in suburban and rural villages in Laos and Thailand

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Abstract

Introduction: Mortality due to diarrhoea in children under-five decreased with about 50% globally between 1990 and 2013. Understanding the risk factors is critical for managing addressing diarrhoeal disease risk in a cost-effective manner. This study aimed to assess diarrhoeal incidence rate, risk factors and their contribution to diarrhoeal disease.

Methods: A two-year longitudinal study was conducted from 2011-2013 in suburban and rural villages in Laos and Thailand, respectively. The study involved 2,007 individuals and representing 3911 person-years of follow-up. Baseline household information on sociodemography, drinking water sources, sanitation and hygiene was obtained through semistructured interviews of household heads and observations. Diarrhoeal incidence data was obtained weekly through individual household members using WHO definition and compared with the countries' national surveillance data. Logistic regression was used to assess the risk factors of diarrhoea and their individual contribution to diarrhoea based on the estimated Population Attributable Fractions (PAF).

Results: A total of 97 diarrhoeal cases were recorded (suburban Laos: 35 cases, rural Laos: 11 cases, suburban Thailand: 12 cases and rural Thailand: 39 cases). The recorded diarrhoeal incidence rate was higher than the official data of both countries, where the highest rate was reported in rural Thailand and suburban Laos with 46.5 and 31.8 followed by suburban Thailand and rural Laos with 15.3 and 9.3 episodes per 1000 person-years, respectively. In rural Thailand and suburban Laos, the most affected age group was children under-five which accounted for 180.0 and 170.5 episodes per 1000 person-years, respectively. Hygiene and socio-demographic factors were more likely to be associated with diarrhoeal cases than other factors. In rural Thailand, households who did not cleaned utensils immediately after eating or cooking increased the risk of diarrhoea (OR. 2.6 [1.1-6.6]) (PAF: 53%). In suburban Laos, factors associated with diarrhoea were houses with utensils left unwashed (Odd ratio. 2.4 [1.1-5.4]) (PAF: 11%) and disposal of baby stool in the open (OR. 3.9 [1.1-15.5]) (PAF: 8%).

Conclusion: Diarrhoeal incidence was higher than the national surveillance data especially in children under-five. Health messages on proper disposal of stool from infants and increasing the numbers and use of toilets in Laos would enhance environmental hygiene. Furthermore, better kitchen hygiene and cleaning of utensils after eating or cooking is important both in the Lao and Thai villages.

Key words: Diarrhoeal incidence, follow up, Laos, risk factor, Thailand

Introduction

Over the past decades, progress made in water, sanitation and hygiene globally have resulted in a significant reduction in diarrhoeal diseases (WHO 2015a). However, diarrhoeal disease due to poor water, sanitation and hygiene practices still remains a major challenge (WHO 2014). Children under-five years are particularly disproportionately affected by the burden of diarrhoeal disease. Globally, diarrhoeal disease in children under-five years of age is a major public health concern and ranked as the second leading cause of death (WHO 2013a). In Laos and Thailand, diarrhoea within this age group ranked as the 5th and 8th leading cause of child mortality, respectively (WHO 2013b, 2013c). This corresponds to 11 and 3% of child mortality in Laos and Thailand respectively (WHO 2015b).

Reports from the National Center for Laboratory and Epidemiology in Laos showed an increased incidence in acute watery diarrhoeal disease cases from 215/100,000 in 2009 to 481/100,000 people in 2013 (Houatthongkham *et al.* 2016). In Thailand, the annual incidence rates over the same period revealed a decreasing trend from 2,024/100,000 in 2009 to 1,765/100,000 people in 2013 (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. Diarrhoea).

Enteric bacterial, viral and parasitic pathogens may cause diarrhoeal diseases after oral transmission through fecal contamination, from person to person or as a result of poor hygiene (WHO 2005). Diarrhoeal etiological studies conducted in Laos and Thailand in the 1990ies showed the dominance of bacterial pathogens like *Shigella spp, E. coli* and Campylobacter spp as the major etiological agents among patients visiting medical facilities (Echeverria *et al*. 1994; Yamashiro *et al.* 1998). More recent studies found that viral pathogens like Rotavirus accounted for a high proportion of acute gastroenteritis in these countries (Aloun *et al.* 2009; Chaimongkol *et al.* 2012; Platts-Mills *et al.* 2015; Houatthongkham *et al.* 2016). Rotavirus vaccine is still not part of the National Immunization Program in Laos and Thailand. There are also no coordinated targeted interventions for diarrhoeal disease within the household environments of both countries. For the development of cost-effective interventions, it is pertinent that the risk factors associated with diarrhoeal disease are identified and their specific contributions to diarrhoeal disease ascertained. This will allow for the judicious allocation of resources to achieve the maximum impact in terms of the reduction of diarrhoeal disease burden. The overall aim of this study was to assess diarrhoeal incidence rate, risk factors and their attributable risk for diarrhoeal disease in suburban and rural villages in Laos and Thailand.

Methods

Study areas and participants

The study was conducted in one suburban and one rural village in Laos (at Lakhonpheng district, Salavan province) and Thailand (at Manchakhiri district, Khon Kaen province), respectively (Figure 1). Initially, the study started to follow a total number of 478 households comprising 2,035 individuals selected based on a systematic sampling of houses in each village. At the end of the study the sample size had reduced to 471 households comprising 2,007. This change was accounted for by loss to follow-up and new additions to the sampled households (new born babies and new individuals moving in). A detailed breakdown on these changes is presented in Figure 1.

Study design

A cross-sectional survey was conducted to collect information on the households' sociodemographic characteristics, drinking water sources, sanitation and hygiene. Information on diarrhoeal disease incidence was collected through a two-year longitudinal survey. These surveys are described as follows:

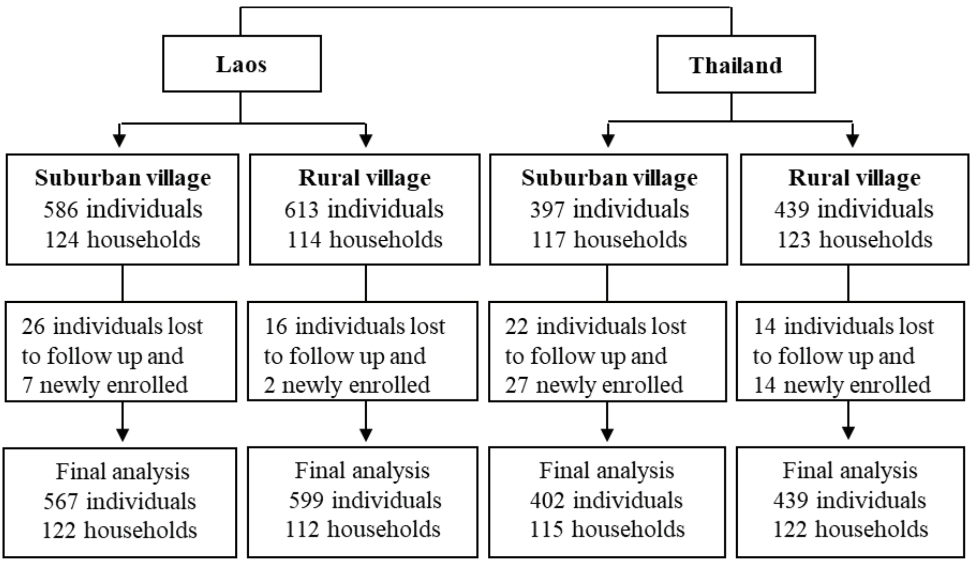


Figure 1. Number of participants and households during the two-year follow up period in four villages

Household socio-demographic characteristics, drinking water, sanitation and hygiene

General information on socio-demographic characteristics, drinking water sources, sanitation and hygiene was collected through semi-structure interviews with the household heads as well as observations. The socio-demographic characteristics information collected included age, gender, education, occupation, housing materials, room occupancy rate and socioeconomic status (SES). Information drinking water included the type of drinking water and source (e.g. rainwater, purchased bottled water or water from boreholes), household's water treatment method and mode of water collection. Information about type of sanitation facility, open defaecation practice, and hygienic behavior of the people were also collected. Information about handwashing facility with soap, handwashing after toilet use was also collected. Additionally, presence of pets (dog, cat) at home, presence of animal/human faeces on yard, mode of infant stool disposal, kitchen utensils left unwashed after use or cleaning directly after eating/cooking and presence of houseflies in kitchen were observed and accounted for in the survey.

Weekly record of diarrhoeal disease

Door-to-door visits was conducted weekly by trained village health volunteers during the two years study to record the diarrhoeal cases that occurred among the household members in all the selected households in the villages. This initiation time was different depending on the sites (suburban Laos: May 2011-March 2013, rural Laos: June 2011-April 2013, suburban Thailand: April 2011-February 2013, and rural Thailand: March 2011-January 2013). Diarrhoea was defined as the passage of three or more loose or liquid stools within 24 hours (WHO 2005). A new episode of diarrhoea was considered when it was re-occurring at least three days after the first diarrhoea had stopped (Baqui *et al.* 1991). If the case was present during the visit the general information of a patient were collected, through a questionnaire form, directly from the case or from mother/caretaker if one was less than 15 years old. The secondary data of diarrhoeal disease during the same period at the district level of the study villages were also obtained from the national surveillance system of Laos and Thailand. In this study, efforts were made to collect stool samples from the diarrhoeal cases. However, this was truncated because the cases and caretakers were consistently not able to provide the stool samples.

Consent and ethics

Written informed consents were obtained from both the household heads and the diarrhoeal cases. Research ethics was approved by the National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (No. 03/NECHR) on 17 December 2010 and by the Ethical Committee of Phramongkutklao College of Medicine, Bangkok, Thailand (S033h/53) on 21 March 2011.

Data analysis

Descriptive analysis of socio-demographic characteristics, drinking water sources, sanitation and hygiene were done for each study village. Further analysis was undertaken to derive additional risk factors such as room occupancy rate and wealth status of the households. Estimation of the room occupancy rate was based on United Nation's definition (United Nations Statistics Division 2016). Wealth status of the households were ranked into rich, intermediate and poor using Principal Components Analysis (PCA) based on group weighted mean scores (Vyas and Kumaranayake 2006). In addition, general information of the diarrhoeal cases was also displayed including their personal profiles, type of diarrhoea, diarrhoeal treatment and place of the treatment.

Diarrhoeal incidence rate was measured as a diarrhoeal episode per 1000 person-years (Calculating Person-Time 2015). To measure the incidence rate, the actual time in years of each household member was used and the diarrhoeal event summed up from the onset to the termination year. The person-years-at-risk was used as a denominator and the total numbers of diarrhoeal cases in each village derived from two-year record were used as a numerator, and the outcomes multiplied with a population of 1000. During the follow-up time, the newborn babies together with other people who were not initially registered and have moved into the houses were considered as newly enrolled observations, while those who were lost to the follow up were excluded. The total period of the lost followed individuals that spent time in the respective village during the study was also included in the calculation of the incidence rate.

Univariate and multivariate logistic regression models were used to find significant relationship between diarrhoea and different risk factors in each village. The variables with a significance level of $p \leq 0.2.5$ derived from a univariate analysis were included in the multivariate model. Backward stepwise selection procedure was used to obtain significant risk factors ($p<0.05$) from the multivariate analysis.

To measure the public health impact of several diseases including diarrhoea, Population Attributable Fraction (PAF) was used as a quantifiable tool (Genser *et al.* 2008; Seidu *et al.* 2013). It has further been used to quantify the contribution of different risk factors to the burden of diseases (WHO 2017). All significant risk factors obtained from the multivariate analysis were then used to calculate PAF by using the following formula:

$$\mathbf{PAF} = \frac{\mathbf{Ppop\ x\ (RR-1)}}{\mathbf{Ppop\ x\ (RR-1)+1}}$$

where P_{pop} is the proportion of exposed subjects and RR is the relative risk or rate ratio (Flegal *et al.* 2015). All statistical analyses were conducted using STATA (version 12; STATA Corporation, College Station, TX, USA).

Results

General information of households and household members

The background information on the characteristics of the included villages and the selected households are given in Table 1. The Thai villagers had a better wealth status than the Lao villagers. The proportion of poor households in rural Laos was 81% while 66% of the suburban Thai villagers fall within the "rich household" category. The mean age of the people in suburban and rural Laos was 25.7 and 28.1 years while for the Thai villages the corresponding figures were 40.2 and 41.6 years, respectively. Most people in rural villages only had primary school education while people with higher education were found in suburban villages. The main occupation was within agriculture especially in the rural villages (65% for each). About 58% and 54% of the households in suburban Laos and Thailand relied on drinking water from purchased bottled water, respectively, while rainwater from rain-fed and manually collected rainwater was the main drinking water source in rural villages (91% and 98% in rural Laos and Thailand, respectively). Of these, 71% and 89% of the households in rural villages respectively did not treat or boil water before drinking. All Thai villages had access to sanitation facility which was not the case in Laos. In suburban and rural Laos, 21% and 78% of the households lacked access to the toilets, of which 16% and 80% of those lacking access practiced opened defaecation, respectively.

Table 1. General information of households and household members in a suburban and a rural village in Laos and Thailand (percentages in parentheses)

		Laos		Thailand	
		Suburban	Rural	Suburban	Rural
No. of individuals		**567**	**599**	**402**	**439**
Age group	0-5	76 (13.4)	57 (9.5)	22 (5.5)	30 (6.9)
	>5-15	134 (23.6)	146 (24.4)	64 (15.9)	44 (10.0)
	>15	357 (63.0)	396 (66.1)	316 (78.6)	365 (83.1)
Mean age		25.7	28.1	40.2	41.6
Gender	Male	274 (48.3)	305 (50.9)	188 (46.8)	221 (50.3)
	Female	293 (51.7)	294 (49.1)	214 (53.2)	218 (49.7)
Education	Primary school	186 (32.8)	427 (71.3)	196 (48.8)	295 (67.2)
	Junior secondary school	122 (21.5)	52 (8.7)	46 (11.4)	37 (8.4)
	Senior secondary school	60 (10.6)	26 (4.3)	64 (15.9)	49 (11.2)
	Tertiary	83 (14.6)	10 (1.7)	70 (17.4)	16 (3.6)
	Other (elderly, children)	116 (20.5)	84 (14.0)	26 (6.5)	42 (9.6)
Occupation	Agriculture	178 (31.4)	392 (65.4)	50 (12.4)	284 (64.7)
	Service	93 (16.4)	19 (3.2)	27 (6.7)	14 (3.2)
	Commerce	33 (5.8)	12 (2.0)	80 (19.9)	8 (1.8)
	Unemployed	8 (1.4)	3 (0.5)	53 (13.2)	9 (2.1)
	Student	146 (25.8)	102 (17.0)	89 (22.1)	62 (14.1)
	Other*	109 (19.2)	71 (11.9)	103 (25.6)	62 (14.1)
No. of households		**122**	**112**	**115**	**122**
Room occupancy rate	> 2.5 persons/room	61 (50.0)	43 (38.4)	21 (18.3)	32 (26.2)
	≤ 2.5 persons/room	61 (50.0)	69 (61.6)	94 (81.7)	90 (73.8)
Wealth status	Poor	38 (31.1)	91 (81.2)	5 (4.3)	19 (15.6)
	Intermediate	51 (41.8)	15 (13.4)	34 (29.6)	66 (54.1)
	Rich	33 (27.1)	6 (5.4)	76 (66.1)	37 (30.3)
Housing material	Cement	24 (19.7)	1 (0.9)	23 (20.0)	16 (13.1)
	Cement-wood	48 (39.3)	16 (14.3)	80 (69.6)	74 (60.7)
	Wood	50 (41.0)	95 (84.8)	12 (10.4)	32 (26.2)
Drinking water source (most used)†	Rain-fed	9 (7.5)	17 (15.2)	24 (20.9)	66 (55.0)
	Manually collected rain	18 (15.0)	85 (75.9)	26 (22.6)	51 (42.5)
	Purchased bottled water	69 (57.5)	10 (8.9)	62 (53.9)	2 (1.7)
	Borehole	23 (19.2)	0	0	0
Water treatment	No	22 (18.0)	79 (70.4)	40 (34.8)	109 (89.3)
	Yes	100 (82.0)	33 (29.6)	75 (65.2)	13 (10.7)
Sanitation facility	Pour flush toilet	70 (57.4)	23 (20.5)	96 (83.5)	118 (96.7)
	Flush toilet	7 (5.7)	1 (0.9)	17 (14.8)	0
	Shared toilet	19 (15.6)	1 (0.9)	2 (1.7)	4 (3.3)
	No toilet	26 (21.3)	87 (77.7)	0	0
Open defaecation	No	103 (84.4)	22 (19.6)	115 (100)	122 (100)
	Yes	19 (15.6)	90 (80.4)	0	0
Mode of stool disposal of the baby/children who don't use the toilet	Rinse into toilet	40 (32.8)	11 (9.8)	11 (9.6)	13 (10.7)
	Put into garbage	7 (5.7)	0	6 (5.2)	2 (1.6)
	Buried	26 (21.3)	10 (8.9)	1 (0.9)	1 (0.8)
	Left in the open	4 (3.3)	1 (0.9)	0	0
	Other (no child at home)	45 (36.9)	90 (80.4)	97 (84.3)	106 (86.9)
Utensils left unwashed in kitchen	No	105 (86.1)	109 (97.3)	64 (55.7)	62 (50.8)
	Yes	17 (13.9)	3 (2.7)	51 (44.3)	60 (49.2)
Cleaning utensil after	Immediately	52 (42.6)	101 (90.2)	27 (23.5)	34 (27.9)
	Next cooking/day	70 (57.4)	11 (9.8)	88 (76.5)	88 (72.1)

eating/cooking

† Two households in each suburban Laos and rural Thailand were missing of drinking water sources
*Other: retired and children

Diarrhoeal cases and incidence rates

The numbers of diarrhoeal cases obtained from the weekly records during the two years are presented in Table 2. Overall, 97 diarrhoeal cases with no death were recorded which included 35 and 11 cases from suburban and rural Laos respectively; and 12 and 39 cases for suburban and rural Thailand villages, respectively. Of the reported diarrhoeal disease cases, acute watery diarrhoea accounted for 86% and 73% of the cases in suburban and rural Laos, and 100% and 92% of the cases in suburban and rural Thailand, respectively. In Laos, the majority of cases were found in the suburban village (35 cases, with 2 recurrent infections), of which, 63% were children ≤5 years. In Thailand, most of the cases were found in the rural village (39 cases, with 7 recurrences), with 69% of the cases accounted for by household members older than 15 years (Table 2).

In Laos 94% and 100% of the diarrhoeal cases in suburban and rural villages respectively received treatment. In Thailand, 85% and 92% of the diarrhoea cases in surburban and rural villages received treatment, respectively. Among those receiving treatment, 60% of cases in suburban Laos and 50% and 55% in rural and suburban Thailand respectively received a medical treatment at the health facility. No health facility was available in the rural village of Laos and the individuals relied either on village health workers or self-medication.

Table 2. Numbers of diarrhoeal cases from the two-year follow up in a suburban and a rural village in Laos and Thailand (percentages in parentheses)

		Laos		Thailand	
		Suburban	Rural	Suburban	Rural
No. of diarrhoea		**35***	**11**	**12**	**39†**
Age group	0-5	22 (62.8)	2 (18.2)	1 (8.3)	9 (23.1)
	>5-15	3 (8.6)	1 (9.1)	2 (16.7)	3 (7.7)
	>15	10 (28.6)	8 (72.7)	9 (75.0)	27 (69.2)
Gender	Male	18 (51.4)	6 (54.6)	3 (25.0)	22 (56.4)
	Female	17 (48.6)	5 (45.4)	9 (75.0)	17 (43.6)
Type of diarrhoea	Acute watery diarrhoea	30 (85.7)	8 (72.7)	12 (100)	36 (92.3)
	Dysentery	5 (14.3)	3 (27.3)	0	3 (7.7)
Diarrhoea	No	2 (5.7)	0	1 (8.3)	6 (15.4)
treatment	Yes	33 (94.3)	11 (100)	11 (91.7)	33 (84.6)
Treatment places	Health facility	20 (60.6)	0	6 (54.6)	17 (50.0)
	Private clinic	7 (21.2)	2 (18.2)	0	2 (5.9)
	Health worker	4 (12.1)	5 (45.4)	0	2 (5.9)
	Self-medication	2 (6.1)	4 (36.4)	5 (45.4)	13 (38.2)

* There are 2 recurrent infections, † There are 7 recurrent infections

The incidence rates of diarrhoeal disease per 100,000 people recorded in this study is shown in Figure 2. The highest incidence was reported in rural Thailand in 2011 and in suburban Laos in 2012 with 6150.3 and 3527.3 cases per 100,000 people, respectively (Figure 2). In Laos, most of the diarrhoea cases were recorded in the dry season (February-March) (Figure 3), while in Thailand most of the diarrhoea cases were recorded during rainy season (August-December) (Figure 3).

In addition, the incidence rate of diarrhoeal episodes per time is presented in Table 3. Of the 2,007 individuals, the person-years during the two-year follow-up times for each village are given. The highest diarrhoeal incidence rate was recorded in rural Thailand and suburban Laos with 46.5 and 31.8 episodes per 1000 person-years, respectively. Among these, the most affected age group was the children under-five which accounted for up to 180.0 and 170.5 episodes per 1000 person-years in rural Thailand and suburban Laos, respectively. The lowest diarrhoeal incidence was recorded in rural Laos and suburban Thailand with 9.3 and 15.3 episodes per 1000 person-years).

According to the district level secondary diarrhoeal incidence data from 2010 to 2012, Thailand reported a higher diarrhoeal incidence per 100,000 populations than Laos (Figure 4). In our findings, the incidence rates of diarrhoeal disease recorded in both suburban Laos and rural Thailand (Figure 2).

Figure 2. Diarrhoeal incidence in suburban and rural villages in Laos and Thailand (2011-2013). Figures represent the number of diarrhoeal cases per 100,000 populations

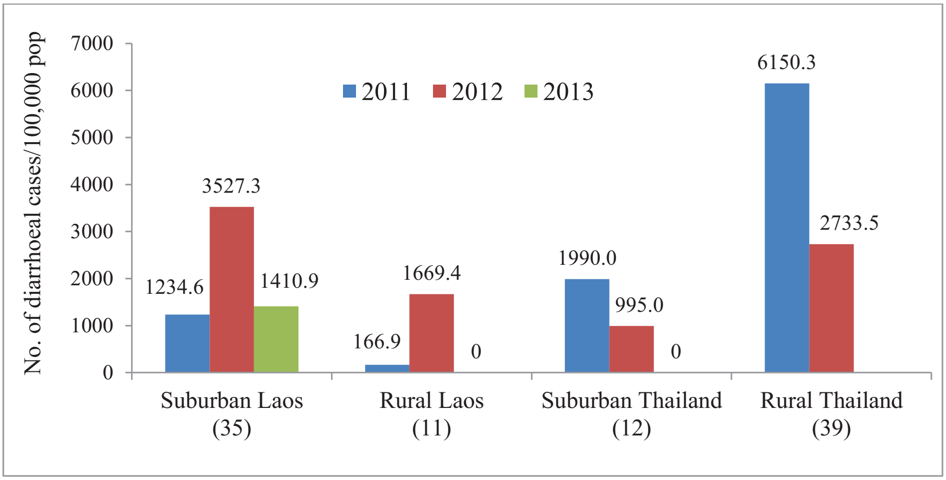


Figure 3. Temporal distribution of diarrhoeal disease in suburban and rural villages in Laos (n=46) and Thailand (n=51). Figures in brackets indicate number of cases

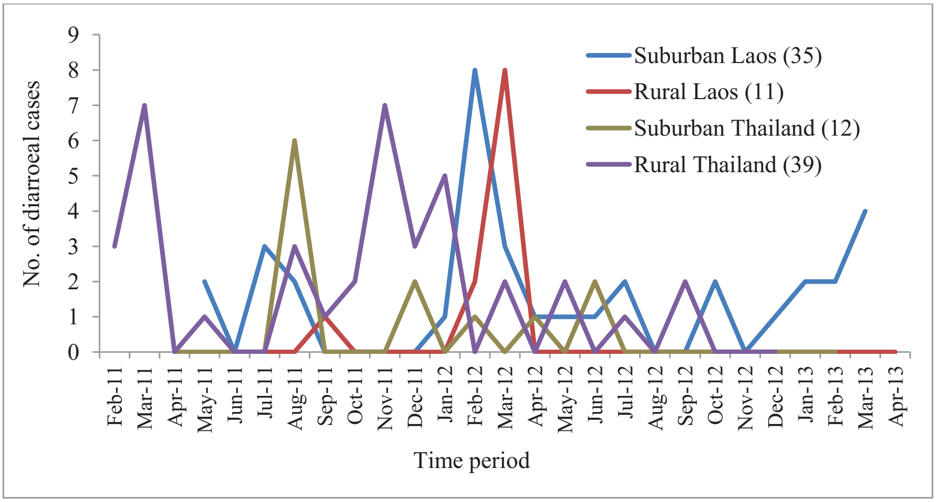
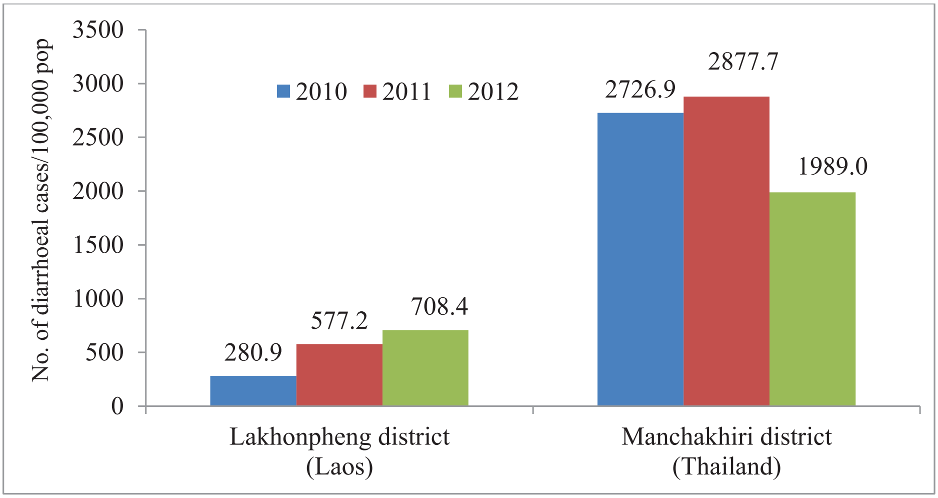


Figure 4. Diarrhoeal incidence in Lakhonpheng district of Laos and in Manchakhiri district of Thailand (2010-2012). Figures represent the number of diarrhoeal cases per 100,000 populations reported in the districts in Laos and Thailand where the study villages are located (secondary data derived from the national surveillance system)



Diarrhoeal risk factors and Population Attributable Fraction (PAF)

Results of the univariate and multivariate analyses on the relationship between the risk factors and diarrheoal disease are presented in Tables 4 and 5, respectively. The risk factors were mainly associated with hygiene and socio-demographic characteristics rather than drinking water sources. The significant risk factors associated with the occurrence of diarrhoea in suburban Laos were children ≤5 years (OR. 14.9 [6.4-34.4]) as compared to those older than15 years of age (used as a reference), and those who disposed of stool of children in the open (OR. 3.9 [1.1-15.5]) compared to those who disposed it into a toilet. Households who left utensils unwashed in the kitchens were also at higher risk compared with those who washed utensils (OR. 2.4 [1.1-5.4]). Individuals in suburban Laos living in houses made from both cement and wood as compared to cemented houses were 3 times as likely to develop diarrhoea as those living in other houses, but the relationship was not significant on the 95% level (p =0.079) (Table 5). Similarly, the risk group of diarrhoea found in rural Thailand were children ≤5 years of age (OR. 3.7 [1.7-8.2]), and those who left utensils uncleaned after cooking/eating until the next cooking time or next day (OR. 2.6 [1.1 - 6.6]) compared to immediate cleaning. In rural Laos, the presence of animal/human faeces in the yard increased the risk of diarrhoeal disease (OR. 9.3 [1.2-74.4]). In suburban Thailand, diarrhoeal disease risk was associated with the presence of flies in the kitchen of the household (OR. 12.6 [3.5-45.2]), and in households with the room occupancy rate of ≤2.5 (p=0.095).

The population attributable fractions (PAFs) related to the diarrhoeal risk factors are shown in Table 6. The PAF values were attributed to diarrhoea in each village and most were related to hygiene and socio-demographic characteristics as earlier stated for the univariate and multivariate analyzes. In Laos, diarrhoeal disease cases were attributed to the presence of children under-five years of age in the suburban site (PAF: 58%), which was not the case for the other villages. In Thailand, the highest PAF attributed to diarrhoea was a delay in cleaning utensils (53%) in the rural village. In the rural Laos and suburban Thailand where low numbers of diarrhoeal cases were reported, 80% and 29% of the cases were respectively attributed to the presence of animal or human faeces in the yard and with the presence of flies at the kitchen.

Table 3. Diarrhoeal incidence per 1000 person-years in a suburban and a rural village in Laos and Thailand

		Laos						Thailand					
		Suburban (n= 567)			**Rural (n= 599)**			**Suburban (n= 402)**			**Rural (n= 439)**		
		Case	person years	Incidence Rate	Case	person years	Incidence Rate	Case	person years	Incidence Rate	Case	person years	Incidence Rate
Age group	0-5	22	129	170.5	2	111	18.0	1	42	23.8	9	50	180.0
	>5-15	3	264	11.4	1	291	3.4	2	124	16.1	3	87	34.5
	>15	10	709	14.1	8	784	10.2	9	619	14.5	27	701	38.5
Total		**35**	**1102**	**31.8**	**11**	**1186**	**9.3**	**12**	**785**	**15.3**	**39**	**838**	**46.5**

n: no. of individuals

Table 4. Univariate analyses of risk factors associated with diarrhoeal disease in a suburban and a rural village in Laos and Thailand (Odds ratio (OR) ([95% confidence intervals, CI] p-value)

		%	Suburban Laos (n= 567)	%	Rural Laos (n= 599)	%	Suburban Thailand (n= 402)	%	Rural Thailand (n= 439)
Socio-demography									
Age group	>15	63	1	66	1	79	1	83	1
	>5-15	24	1.2 [0.4-3.8] 0.779	24	0.3 [0.0-2.7] 0.308	16	1.1 [0.2-5.1] 0.906	10	1.2 [0.4-3.4] 0.732
	0-5	13	11.5 [5.3-25.0]< 0.001	10	1.7 [0.4-8.2] 0.485	5	1.6 [0.2-12.6] 0.657	7	3.7 [1.7-8.2] 0.001
Gender	Male	48	1	51	1	47	1	50	1
	Female	52	0.8 [0.4-1.5] 0.481	49	0.9 [0.3-2.8] 0.810	53	2.6 [0.7-9.7] 0.146	50	0.8 [0.4-1.5] 0.449
Education	≤ Primary school	53	1	85	1	55	1	77	1
	> Primary school	47	0.2 [0.1-0.5] 0.001	15	0.6 [0.1-4.5] 0.604	45	0.6 [0.2-2.0] 0.430	23	1.7 [0.8-3.2] 0.139
Room occupancy rate	> 2.5 persons/room	56	1	50	1	27	1	70	1
	≤ 2.5 persons/room	44	0.7 [0.3-1.3] 0.238	50	0.8 [0.3-2.7] 0.751	73	1.9 [0.4-8.5] 0.423	30	1.9 [1.1-3.7] 0.034
Housing material	Cement	18	1	1	1	21	1	12	1
	Cement-wood	42	2.4 [0.7-8.1] 0.166	13	-	66	0.9 [0.3-3.5] 0.926	63	0.7 [0.3-1.8] 0.480
	Wood	40	2.2 [0.6-7.5] 0.217	86	-	13	-	25	0.9 [0.3-2.4] 0.822
Wealth status	Poor	29	1	82	1	4	1	14	1
	Intermediate	42	1.3 [0.6-2.9] 0.494	11	0.7 [0.1-5.6] 0.753	34	-	53	0.8 [0.3-1.7] 0.546
	Rich	29	1.0 [0.4-2.6] 0.949	7	-	62	-	33	0.4 [0.2-1.2] 0.098
Drinking water source and management †									
Rain-fed	No	93	1	86	1	80	1	44	1
	Yes	7	-	14	1.3 [0.3-6.3] 0.692	20	2.8 [0.9-8.9] 0.076	56	0.8 [0.4-1.6] 0.609
Manually collected rain	No	87	1	26	1	76	1	58	1
	Yes	13	0.9 [0.3-2.5] 0.821	74	1.5 [0.3-7.1] 0.579	24	-	42	1.3 [0.7-2.5] 0.393
Purchased bottled water	No	41	1	88	1	47	1	98	1
	Yes	59	1.2 [0.6-2.5] 0.552	12	-	53	1.2 [0.4-3.9] 0.711	2	-
Borehole	No	80	1	100	1	100	1	100	1
	Yes	20	1.1 [0.5-2.5] 0.874	0	-	0	-	0	-
Water treatment	No	18	1	70	1	36	1	91	1
	Yes	82	1.3 [0.5-3.4] 0.569	30	0.2 [0.0-1.8] 0.165	64	0.6 [0.2-1.8] 0.331	9	0.8 [0.3-2.7] 0.758
Mode of collecting	Pouring into cup	39	1	35	1	65	1	31	1

water to drink	Scooping into cup	61	1.2 [0.6-2.3] 0.669	65	0.4 [0.1-1.9] 0.264	35	1.1 [0.3-3.5] 0.914	69	0.8 [0.4-1.5] 0.459
Sanitation and hygiene									
Sanitation ladder	Pour flush toilet	59	1	20	1	81	1	97	1
	Flush toilet	6	0.9 [0.2-3.8] 0.870	1	-	14	0.5 [0.1-3.9] 0.518	0	-
	Shared toilet	19	0.7 [0.3-1.9] 0.555	1	-	5	-	3	-
	No toilet	16	1.3 [0.5-2.9] 0.603	78	0.7 [0.2-2.6] 0.583	0	-	0	-
Open defaecation	No	84	1	19	1	100	1	100	1
	Yes	16	1.3 [0.6-3.1] 0.485	81	1.0 [0.2-4.7] 0.976	0	-	0	-
Hand washing facility with soap near or inside the toilet									
	No	31	1	77	1	16	1	1	1
	Yes	69	1.5 [0.7-3.3] 0.299	23	1.3 [0.3-4.7] 0.739	84	2.1 [0.3-16.4] 0.471	99	-
Hand washing after the toilet	Sometime	13	1	11	1	14	1	11	1
	Always	87	0.9 [0.4-2.4] 0.853	89	0.5 [0.1-2.4] 0.415	86	0.5 [0.1-1.8] 0.265	89	0.8 [0.3-2.1] 0.670
Having pet at home	No	14	1	12	1	45	1	33	1
	Yes	86	1.0 [0.4-2.6] 1.000	88	1.4 [0.2-10.8] 0.755	55	1.6 [0.5-5.4] 0.420	67	0.8 [0.4-1.5] 0.434
Animal/human faeces on yard	No	12	1	45	1	81	1	82	1
	Yes	88	1.0 [0.4-2.9] 0.943	55	8.2 [1.1-64.1] 0.045	19	-	18	0.5 [0.2-1.5] 0.216
Mode of stool disposal of the baby/children who don't use the toilet	Rinse into toilet	36	1	10	1	13	1	12	1
	Put into garbage	6	3.2 [1.1-9.5] 0.037	0	-	10	1.3 [0.1-20.4] 0.864	3	0.5 [0.1-4.1] 0.541
	Buried	20	1.6 [0.6-4.0] 0.364	9	3.5 [0.4-33.7] 0.277	1	-	1	-
	Left in the open	4	3.2 [0.9-11.8] 0.082	1	-	0	-	0	-
	No baby/children	34	1.1 [0.5-2.8] 0.768	80	0.9 [0.1-7.4] 0.926	76	1.7 [0.2-12.9] 0.631	84	0.5 [0.2-0.9] 0.037
Utensils left unwashed in kitchen	No	84	1	98	1	55	1	50	1
	Yes	16	1.8 [0.8-3.8] 0.133	2	4.2 [0.5-32.7] 0.173	45	2.5 [0.8-8.3] 0.136	50	1.0 [0.6-1.9] 0.906
Cleaning utensil after eating/cooking	Immediately	41	1	90	1	19	1	28	1
	Next cooking/day	59	1.7 [0.8-3.6] 0.148	10	0.9 [0.1-7.0] 0.919	81	2.6 [0.3-20.2] 0.359	72	2.6 [1.1-6.6] 0.047
Presence of housefly in kitchen	No	96	1	100	1	94	1	99	1
	Yes	4	2.4 [0.7-7.9] 0.140	0	-	6	7.6 [2.3-25.1] 0.001	1	-

† Source of drinking water was missing in two households of suburban Laos and rural Thailand
n: no. of individuals

Table 5. Multivariate analyses of risk factors associated with diarrhoeal disease in a suburban and a rural village in Laos and Thailand (Odds ratio (OR) ([95% confidence intervals, CI] p-value)

		%	Suburban Laos (n= 567)	%	Rural Laos (n= 599)	%	Suburban Thailand (n= 402)	%	Rural Thailand (n= 439)
Socio-demography									
Age group	>15	63	1					83	1
	>5-15	24	1.3 [0.4-4.2] 0.683					10	1.2 [0.4-3.3] 0.793
	0-5	13	**14.9 [6.4-34.4]< 0.001**					7	**3.7 [1.7-8.2] 0.001**
Room occupancy rate	> 2.5 persons/room					27	1		
	≤ 2.5 persons/room					73	3.9 [0.8-20.0] 0.095		
Housing material	Cement	18	1						
	Cement-wood	42	3.1 [0.9-10.7] 0.079						
	Wood	40	1.7 [0.5-6.1] 0.393						
Hygiene									
Animal/human faeces on yard	No			45	1				
	Yes			55	**9.3 [1.2-74.4] 0.035**				
Mode of stool disposal of the baby/children who don't use the toilet	Rinse into toilet	36	1						
	Put into garbage	6	2.2 [0.7-6.9] 0.166						
	Buried	20	1.4 [0.5-3.6] 0.535						
	Left in the open	4	**3.9 [1.1-15.5] 0.047**						
	No baby/children	34	2.6 [0.9-7.0] 0.055						
Utensils left unwashed in kitchen	No	84	1	98	1				
	Yes	16	**2.4 [1.1-5.4] 0.043**	2	6.9 [0.9-55.5] 0.067				
Cleaning utensil after eating/cooking	Immediately							28	1
	Next cooking/day							72	**2.6 [1.1-6.6] 0.048**
Presence of housefly in kitchen	No					94	1		
	Yes					6	**12.6 [3.5-45.2] < 0.001**		

n: no. of individuals

Table 6. Population Attributable Fraction (PAF) (%) of diarrhoeal risks in a suburban and a rural village in Laos and Thailand (Odds ratio (OR) ([95% confidence intervals, CI] p-value). Numbers in bold indicate significant associations ($p < 0.05$)

		Laos				Thailand			
		Suburban (n= 567)	PAF	Rural (n= 599)	PAF	Suburban (n= 402)	PAF	Rural (n= 439)	PAF
Socio-demography									
Age group	>15	1						1	
	>5-15	1.3 [0.4-4.2]						1.2 [0.4-3.3]	
	0-5	**14.9 [6.4-34.4]**	**58**					**3.7 [1.7-8.2]**	**15**
Room occupancy rate	> 2.5 persons/room					1			
	≤ 2.5 persons/room					3.9 [0.8-20.0]	39		
Housing material	Cement	1							
	Cement-wood	3.1 [0.9-10.7]	37						
	Wood	1.7 [0.5-6.1]							
Hygiene									
Animal/human faeces	No			1					
on yard	Yes			**9.3 [1.2-74.4]**	**80**				
Mode of stool disposal	Rinse into toilet	1							
of the baby/children	Put into garbage	2.2 [0.7-6.9]							
who don't use the	Buried	1.4 [0.5-3.6]							
toilet	Left in the open	**3.9 [1.1-15.5]**	**8**						
	No baby/children	2.6 [0.9-7.0]	5						
Utensils left unwashed	No	1		1					
in kitchen	Yes	**2.4 [1.1-5.4]**	**11**	6.9[0.9-55.5]	7				
Cleaning utensil after	Immediately							1	
eating/cooking	Next cooking/day							**2.6 [1.1-6.6]**	**53**
Presence of housefly	No					1			
in kitchen	Yes					**12.6 [3.5-45.2]**	**29**		

n: no. of individuals

Discussion

The diarrhoeal incidence and episodes in 139 low and middle income countries have shown a decreasing trend between 1990 and 2010 (Walker *et al.* 2012). Further baseline information is essential especially in suburban and rural areas where safe water and sanitation facilities are limited and hygiene practices are poor. After the two-year survey, 97 diarrhoeal cases were recorded for which two villages accounted for 76% of the diarrhoeal cases (suburban Laos: 35 cases, rural Thailand: 39 cases). In these two villages, children less than five years were the most affected age group (Table 2). The diarrhoeal disease incidence rate in this study was much lower than the incidence rate reported in a review of diarrhoeal cases in South East Asia (29.9 episodes per 100 person-years for adults and older children) (Walker and Black 2010).

The risk factors of diarrhoeal disease were site specific and were socio-demographic, water and sanitation and hygiene related. In suburban Laos, children under-five years were significantly more likely to be affected by diarrhoea (Table 2). Similar findings have been made is in other studies for moderate to severe diarrhoea (Lamberti *et al.* (2012). However, as mentioned earlier, the majority of the cases in both countries did not go to health care facilities for treatment during the course of the diarrhoea episode (Table 2). According to Lamberti *et al.* (2012), only 31.0 % of care-takers for children under-three in South/South-east Asia with diarrhoea do visit health facilities. The challenge of not going for treatment at the health facility could be due to parents or caretakers perception of diarrhoea as a mild symptom associated with a child's physiological development (Pradhipasen *et al.* 1997).

In rural Laos, the low number of diarrhoeal cases (Figure 3) did not reflect the poor sanitation and water situation since the majority of the households did not have any toilet facility and practiced open defaecation and most people drank untreated water (Table 1). This may partly be explained by habitual seasonal changes where during the rainy season, most families preferred to live temporally and periodically at another house in their rice field in order to take care of crops. Also, household members would have suffered from survey fatigue and refuse to report diarrhoea every week as found in a study conducted in Vietnam (Phuc *et al*. 2014).

The study showed that the wealth status of households was not associated with diarrhoea, which corresponds with an earlier study (Yeager *et al.* 1991). The impact of wealth status on diarrhoea was however demonstrated by Genser *et al.* (2008) where 24% and 13% of diarrhoeal cases were respectively attributed to wealth status before and after diarrhoeal intervention. Seidu *et al.* (2013) found a similar impact of wealth status on diarrhoeal disease with a PAF value of 15%. Other findings associated with diarrhoea were poor personal hygiene's condition or uncleanliness around the household compounds. The latter related to improper disposal of infant stools, presence of animal/human faeces within the yard, utensils left unwashed in the kitchen, delayed cleaning of utensils until the next day or next time for food preparation and presence of houseflies in the kitchen (Table 5). A child's stool should be disposed of safely and not be put/rinsed in a drain/ditch, thrown in the garbage and not left or buried in the open (WHO 2006). Unsafe disposal of stool from children under-three is commonly reported from households of many countries (WSP-UNICEF 2014; 2015a, 2015b) even when latrines are available (Lanata *et al.* 1998; Majorin *et al.* 2014). Improper disposal of stool can significantly increase the odds of diarrhoeal episodes among children under-five (Mihrete *et al.* 2014; Cronin *et al.* 2016; Bawankule *et al.* 2017). Other factors such as utensils left unwashed as well as delayed cleaning of utensils and having animal or human excreta in the yard could also be a major route of transmission of diarrhoea. As a result diarrhoea can occur through a direct contact of children with faeces in yards or eating contaminated foodstuffs being transmitted to by the houseflies.

Although the drinking water in the study settings is highly contaminated with *E. coli* as identified in our previous cross-sectional survey (Vannavong *et al.* 2017), no drinking water samples were collected at the time when diarrhoea occurred. Therefore, the relationship between *E. coli* levels in drinking water containers and diarrhoeal disease was not assessed. Studies had found a significant relationship between diarrhoeal disease and the presence of *E. coli* in drinking water (Jensen *et al.* 2004; Levy *et al.* 2012), and *E. coli* levels of $\geq$ 10 CFU/100 mL (Brown *et al.* 2008; Gundry *et al.* 2009).

A major limitation of this study is the collection of a small number of stools during the course of data collection; therefore, the stool samples were not tested for any of diarrhoea-causing bacteria, virus and other parasites. An epidemiological study conducted in the northeastern rural area of Thailand found that 41% of the stool samples carried some enteropathogenic bacteria even most of people had no diarrhoea (Haque *et al.* 1996).

Conclusion

It is concluded that diarrhoea remain a problem for children under-five years of age in a suburban and a rural village in Laos and Thailand. Hygiene and socio-demographic related factors were mainly responsible for diarrhoeal disease transmission in these settings. Health messages on proper disposal of the baby stool should be combined with the provision of toilets particularly in Lao villages. Furthermore, immediate cleaning of utensils after eating or cooking is also important for both Lao and Thai villages. Treatment of drinking water at the household level should be promoted especially in the rural villages of both countries where access to safe water remains insufficient.

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PAPER IV

Nanthasane Vannavong, Razak Seidu, Thor-Axel Stenström, Nsa Dada, Hans Jørgen Overgaard. Dengue-like illness surveillance: a longitudinal survey in suburban and rural communities in Laos and Thailand. *Western Pacific Surveillance and Response Journal* (submitted: 28.9.2017).

Dengue-like illness surveillance: a two-year longitudinal survey in suburban and rural communities in Laos and Thailand

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Abstract

This study aimed to determine dengue-like illness (DLI), dengue virus (DENV) infection and serotypes; and to identify associated risk factors of DLI in relation to socio-demographical and entomological characteristics in one suburban and one rural community in Laos and Thailand, respectively. A two-year longitudinal study was conducted during the inter-epidemic period 2011-2013 and included 2,007 persons in four villages. Entomological surveys, semi-structured interviews of household heads and observations were conducted. Occurrence of DLI was recorded weekly using the dengue definition of the World Health Organization alongside blood sample collections and was compared with national surveillance dengue data. Risk factors of DLI were assessed using logistic regression. The results showed a total of 83 DLI cases (69 in suburban Laos, 11 in rural Thailand, 3 in rural Laos, and none in suburban Thailand). Four of the 83 DLIs were confirmed DENV, two from suburban Laos (both DENV-1) and two from rural Thailand (both DENV-2). Although the number of detected DLIs during the study period was low, DLI incidence was higher in the study compared to the dengue surveillance data in both countries. Risk factors of DLI in suburban Laos were age and occupation. Although the pupal index was high than proposed dengue transmission threshold, it was not associated with DLI. Our findings show the importance of continuous dengue and dengue vector surveillance, which will have significant impact on early detection of dengue and other diseases transmitted by the dengue vector such as chikungunya and Zika.

Key words: *Aedes aegypti*, dengue-like illness, follow up, Laos, Thailand

Introduction

Dengue is a mosquito-borne viral infection prevalent in tropical and subtropical regions. In South-east Asia, one of the largest outbreaks occurred in 2010.[1-3] During this outbreak, 22,929 cases and 46 deaths were recorded in Laos[2] and 116,947 cases and 139 deaths in Thailand.[4] The incidence in Laos was higher than in neighboring countries, with 367 cases per 100,000 persons.[1] The corresponding numbers in Thailand were 177 cases per 100,000 persons, in Vietnam 150 cases per 100,000 persons and in Cambodia 93 cases per 100,000 persons.[2] Salavan province, located in southern Laos was one of the hardest affected areas. In Thailand most cases occurred in Nakhon Si Thammarat province (6,039 cases) in the south of the country.[5] Dengue outbreaks also occurred in Thailand in 2013 with 154,444 and 136 deaths and in 2015 with 144,952 cases and 148 deaths,[5] where DENV-3 (35.7%) and DENV-2 (27.3%) were the most prevalent in 2013[6] and DENV-4 (33.1%) and DENV-3 (32.6%) being the most prevalent in 2015.[7] In Laos, there was also a large outbreak in 2013 with 15 out of 17 provinces reporting dengue at alarming epidemic levels causing 95 deaths from a total of 44,171 cases[8] with DENV-3 and DENV-2 being the most common serotypes this year.[9] However, there was no outbreak in Laos in 2015 corresponding to the Thailand outbreak.

As demonstrated by these figures, dengue outbreaks occur with increasing size and frequency.[1,3,4,5,8] In Laos and Thailand, the number of cases peak during the rainy season, which commonly occurs during May-October.[1,4] Control of dengue in affected settings relies on vector control by distribution of the larvicide temephos as recommended by the World Health Organization (WHO)[3] and space spraying with pyrethroids.[10] A widely occurring challenge for the effective control of dengue vectors using temephos is that people believe that it is a harmful chemical due to its smell.[11] Another challenge for dengue vector control is insecticide resistance in *Aedes aegypti*, the main dengue vector[12] as identified in Thailand[12] and also in Laos.[13] A review on space spraying for the control of adult dengue vectors revealed that this intervention was not sustainable and there was no evidence showing that it was effective in reducing dengue incidence.[14] Recently, a dengue vaccine has been developed and several national regulatory authorities, including Thailand (but not yet

Laos), have approved it for public use.[3,15] However, it is recommended to be used only in areas where the burden of dengue is high.[3]

Dengue-like illness (DLI) has been used to describe cases where the patients have similar clinical manifestations as dengue infection.[3] For example, DLI was recorded in Africa where there was limited access to laboratory facilities for confirmation of dengue and chikungunya[10] and was used to represent dengue in the Republic of Palau in the western Pacific Ocean that also lacked laboratory facilities and rapid diagnosis test kits for confirming dengue.[16] In southern Laos, hospital-based screening of non-malaria febrile illnesses found that dengue was responsible for 30% of the infections.[17] In Thailand, dengue was the third leading cause of acute febrile illnesses in rural areas.[18]

Routine national dengue surveillance systems use passive case detection to record data on number of dengue cases. Active surveillance of dengue and dengue serotypes in non-outbreak settings is rarely conducted. Identifying the presence of dengue virus (DENV) circulating in these settings is necessary for the improvement of early response, control measures and reduction of the dengue burden. The aims of this study were to determine DLI, dengue infections, DENV serotypes; and to identify associated risk factors of DLI in relation to socio-demographical characteristics and mosquito pupal indices in suburban and rural communities in Laos and Thailand during an inter-epidemic period.

Methods

Study areas and design

A two-year longitudinal study was conducted between 2011 and 2013 in Salavan province, southern Laos and in Khon Kaen province, northeastern Thailand. One suburban and one rural village were selected in each country (Figure 1). The two Lao villages are located about six kilometers from each other in Lakhonpheng district. The two Thai villages are located about nine km from each other in Manchakhiri district. These villages were selected based on high earlier dengue incidence: that more than 70% of village households were using domestic water storage containers; and that no ongoing dengue control programs occurred at the time of the survey.[19]

Participants

The total numbers of households in each village were 215 in suburban Laos, 130 in rural Laos, 272 in suburban Thailand and 139 in rural Thailand. Rural Laos had the lowest number of households (130), and this was used as the sample size across all selected villages. For each of the other included villages, which had more than 130 households, the selection was based on a systematic sampling method; first a random house was identified and additional houses were selected based on a fixed interval derived by dividing the total number of households by 130. All individuals residing in the selected households were included. Figure 1 shows the total number of households and people included in the study. During the course of the study, individuals were lost to follow up because they went to study elsewhere, died, or moved out after marriage, whereas households were lost to follow-up due to families moving and settling in other villages or not agreeing to remain in the study. Migrants and new-borne children were included as newly enrolled participants. Overall, the final number of individuals included in the analysis was 2,007 persons.

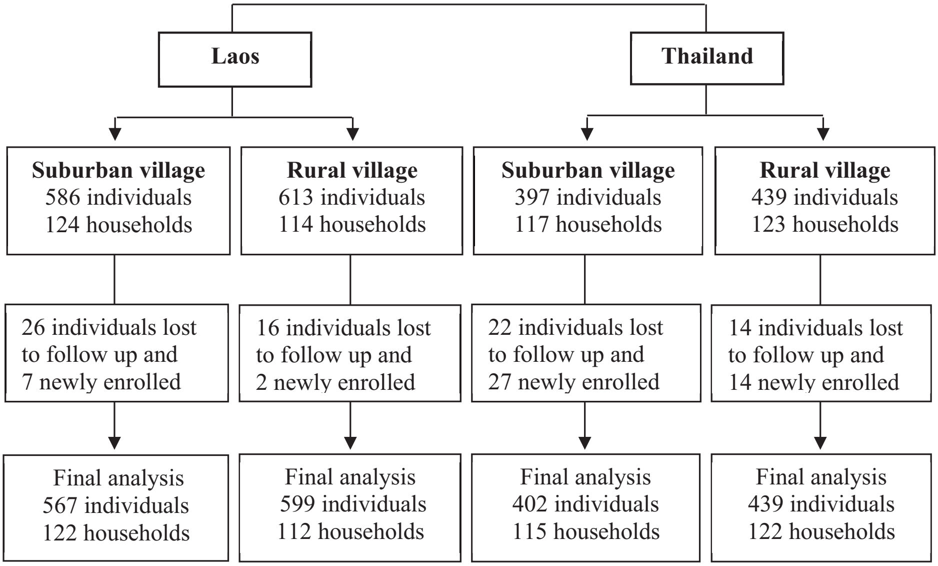


Figure 1. Number of participants and households during the two-year follow up period in four villages

Identification of dengue-like illnesses (DLI)

Households were visited weekly by trained village health volunteers (5-7 persons per village). The study was conducted during May 2011-March 2013 in suburban Laos; June 2011-April 2013 in rural Laos; April 2011-February 2013 in suburban Thailand; and March 2011-January 2013 in rural Thailand. Dengue-like illness was determined based on the dengue definition of the WHO, i.e. presence of acute febrile illness for 2-7 days, with two or more non-specific symptoms, such as headache, retro-orbital pain, myalgia, arthralgia, rash and haemorrhagic manifestations.[20] General information about each case, such as age, gender, education, numbers of days staying at home during the week, sleeping behavior during the day time, and methods of personal protection from mosquito bites, was obtained using an individual questionnaire for patients or guardians if patients were less than 15 years old.

Confirmation of dengue cases

From each identified DLI case, a blood sample was taken by capillary puncture (finger prick) and blotted onto two pieces of filter papers (Blood Sampling Paper, NOBUTO, Toyo Roshi Kaisha Ltd., Japan, Type I) according to the manufacturer's instructions. After the blood was absorbed, the paper was dried at room temperature for 1-2 hours and thereafter tightly sealed in sterile Whirl-Pak Bags. The sealed samples were kept at -20° C until transportation to the processing laboratory. All samples were periodically brought to Thailand where they were further analyzed for DENV RNA confirmation and serotyping using a Real-Time PCR technique as described in Prado *et al* (2005).[21] Secondary data of dengue reported during 2010-2013 were obtained from the national surveillance system in Laos and Thailand for comparison with the numbers of DLI obtained from this study.

Socio-demographic characteristics and entomological survey

General household information was obtained from household heads using a semi-structured household questionnaire. Data collected were age, sex, education level, and occupation of all household members. Additionally, observations and questions regarding housing materials, access to sanitation facilities, and ownership of the durable assets for example mobile or cell phone, TV, radio, refrigerator, car, motorcycle and bicycle were included. All household water storage containers were examined for *Ae. aegypti* pupae, and the number of *Ae. aegypti* pupae, if present, were counted and recorded. Pupae were identified as *Ae. aegypti* using a dissecting microscope and illustrated keys as described in Dada *et al* (2013).[19]

Data analysis

Descriptive analysis of socio-demographic characteristics and entomological data was conducted for each study village. Room occupancy rate was estimated using United Nation's definition.[22] The wealth status of each household was estimated and ranked into rich, intermediate and poor using Principal Components Analysis based on group weighted mean scores.[23] Variables used in the wealth status ranking have been described elsewhere.[24] In addition to socio-demographic characteristics, two entomological indices derived from the entomological survey; pupae per household and pupae per person (number of pupae divided by number of person in each house), were used as potential risk factors of DLI infections.[25] Secondary data of dengue incidence reported from the national surveillance systems in Laos and Thailand were compared with the DLI data obtained in this study. Within and between countries comparisons were conducted using descriptive analysis. A logistic regression was used to find associations between the presence of DLI cases and risk factors. Statistical analyses were carried out using the statistical software SPSS 20.0 (IBM Corp.) and STATA (version 10; STATA Corporation, College Station, TX, USA).

Results

Socio-demographic characteristics

General information of socio-demographics in the study villages is shown in Table 1. The mean ages of people from suburban and rural Laos were 26 and 28 years, while the Thai villagers were older; mean 40 and 42 years, respectively. The main occupation of the people was agriculture especially in the rural villages of both countries where 65% of individuals were farmers. The population densities in the Lao villages based on the room occupancy were more than 2.5 persons per habitable room, which was higher than the Thai sites. In suburban and rural Laos, 41% and 85% of the houses were made from wood, respectively. The houses in the Thai villages were modern and constructed with more durable material such as a mixture of cement and wood (as opposed to wood alone in Laos), accounting for 70% and 61% in suburban and rural village, respectively. The socio-economic status (SES) of the households in Thai villages was generally higher than in Lao villages; the suburban village in Thailand was ranged as the richest (66% of households in the richest SES bracket) whereas rural Laos was the poorest (81% in the poorest SES bracket).

Entomological survey

Infestation of water containers with *Ae. aegypti* pupae was found in all study villages (Table 1). *Aedes aegypti* pupal indices were higher in Thailand than in Laos, with suburban Thailand having the highest pupae per household (8.7) and pupae per person (2.5). Similar pupal indices were recorded in rural Thailand (Table 1). With 5.0 pupae per house and 0.9 pupae per person, rural Laos had the lowest *Ae. aegypti* pupal indices recorded in this study (Table 1).

Table 1. General information of households, household members and dengue-like illnesses (DLI) in selected suburban and rural villages in Laos and Thailand (percentages in parentheses)

		Laos		**Thailand**	
		Suburban	Rural	Suburban	Rural
No. of individuals		**567**	**599**	**402**	**439**
Age group	0-5	76 (13.4)	57 (9.5)	22 (5.5)	29 (6.6)
	>5-10	55 (9.7)	68 (11.3)	25 (6.2)	24 (5.5)
	>10-15	79 (14.0)	78 (13.0)	39 (9.7)	21 (4.8)
	>15-20	67 (11.8)	65 (10.9)	26 (6.5)	34 (7.7)
	>20-25	37 (6.5)	52 (8.7)	19 (4.7)	11 (2.5)
	>25	253 (44.6)	279 (46.6)	271 (67.4)	320 (72.9)
Mean age		25.7	28.1	40.2	41.6
Gender	Male	274 (48.3)	305 (50.9)	188 (46.8)	221 (50.3)
	Female	293 (51.7)	294 (49.1)	214 (53.2)	218 (49.7)
Occupation	Agriculture	178 (31.4)	392 (65.4)	50 (12.4)	284 (64.7)
	Service	93 (16.4)	19 (3.2)	27 (6.7)	14 (3.2)
	Commerce	33 (5.8)	12 (2.0)	80 (20.0)	8 (1.8)
	Unemployed	8 (1.4)	3 (0.5)	53 (13.2)	9 (2.1)
	Student	146 (25.8)	102 (17.0)	89 (22.1)	62 (14.1)
	Other*	109 (19.2)	71 (11.9)	103 (25.6)	62 (14.1)
No. of households		**122**	**112**	**115**	**122**
Room occupancy rate	>2.5 persons/room	61 (50.0)	43 (38.4)	21 (18.3)	32 (26.2)
	≤2.5 persons/room	61 (50.0)	69 (61.6)	94 (81.7)	90 (73.8)
Wealth status	Poor	38 (31.1)	91 (81.2)	5 (4.3)	19 (15.6)
	Intermediate	51 (41.8)	15 (13.4)	34 (29.6)	66 (54.1)
	Rich	33 (27.1)	6 (5.4)	76 (66.1)	37 (30.3)
Housing material	Cement	24 (19.7)	1 (0.9)	23 (20.0)	16 (13.1)

	Cement-wood	48 (39.3)	16 (14.3)	80 (69.6)	74 (60.7)
	Wood	50 (41.0)	95 (84.8)	12 (10.4)	32 (26.2)
***Aedes aegypti* pupal indices†**		**No. [min-max]**	**No. [min-max]**	**No. [min-max]**	**No. [min-max]**
No. of pupae in all positive containers		903	558	1,005	1,005
No. of pupae per household		7.5 [0-102]	5.0 [0-231]	8.7 [0-184]	8.4 [0-153]
No. of pupae per person		1.6 [0-22.5]	0.9 [0-57.8]	2.5 [0-92]	2.3 [0-76.5]
No. of DLI cases		**69**	**3**	**0**	**11**
DLI cases confirmed to dengue		2	0	0	2
DENV serotypes		DENV-1	**-**	**-**	DENV-2
Age group	0-5	4 (5.8)	0	-	0
	>5-10	7 (10.1)	0	-	1 (9.1)
	>10-15	12 (17.4)	0	-	1 (9.1)
	>15-20	12 (17.4)	1 (33.3)	-	1 (9.1)
	>20-25	6 (8.7)	0	-	0
	>25	28 (40.6)	2 (66.7)	-	8 (72.7)
Gender	Male	31 (44.9)	0	-	2 (18.2)
	Female	38 (55.1)	3 (100.0)	-	9 (81.8)
Mean number of days staying in village during the week before DLI detection		6.6	6.3	-	6.6
Sleeping during the day	No	28 (40.6)	0	-	4 (36.4)
	Yes	41 (59.4)	3 (100.0)	-	7 (63.6)
Dengue protection methods	Mosquito net	69 (100.0)	3 (100.0)	-	8 (72.7)
	Bug zapper	11 (15.9)	1 (33.3)	-	0
	Mosquito coil	4 (5.8)	0	-	3 (27.3)
	Insecticide spray	3 (4.4)	0	-	3 (27.3)
	Repellent	2 (2.9)	0	-	1 (9.1)
	Other	5 (7.3)	0	-	4 (36.4)

†*Aedes aegypti* pupal indices applied from Dada et al (2013),[19] number of *Aedes aegypti* pupae was not available in one household of suburban Laos and in two households of rural Thailand
*Other: retired and children, Min-max: minimum-maximum

Dengue-like illnesses (DLI) and confirmed dengue cases

A total of 83 DLI cases were reported during the study period, with 69 (age range: 4-75 years, mean: 25 years, male:female ratio: 0.8) in suburban Laos, 3 in rural Laos (age range: 16-65 years, mean: 49, 3 females), and 11 in rural Thailand (age range: 6-67 years, mean: 42 years, male:female ratio: 0.2). There were no cases recorded in suburban Thailand (Table 1). Of the 83 cases, four were confirmed DENV positive (4.8%); two from suburban Laos (both DENV-1) and two from rural Thailand (both DENV-2) (Table 1).

In suburban Laos, DLI cases were recorded during the entire study period in both 2011 (34 cases) and 2012 (34 cases). The majority of the cases in rural Thailand (10 cases) were recorded in 2011 (Figure 2A). Most of the cases were found during the end of the rainy seasons (August to October in 2011 and October to November in 2012). The confirmed dengue cases were also identified during these time periods. In rural Thailand, the confirmed dengue cases were found in November and December 2011 (Figure 2A).

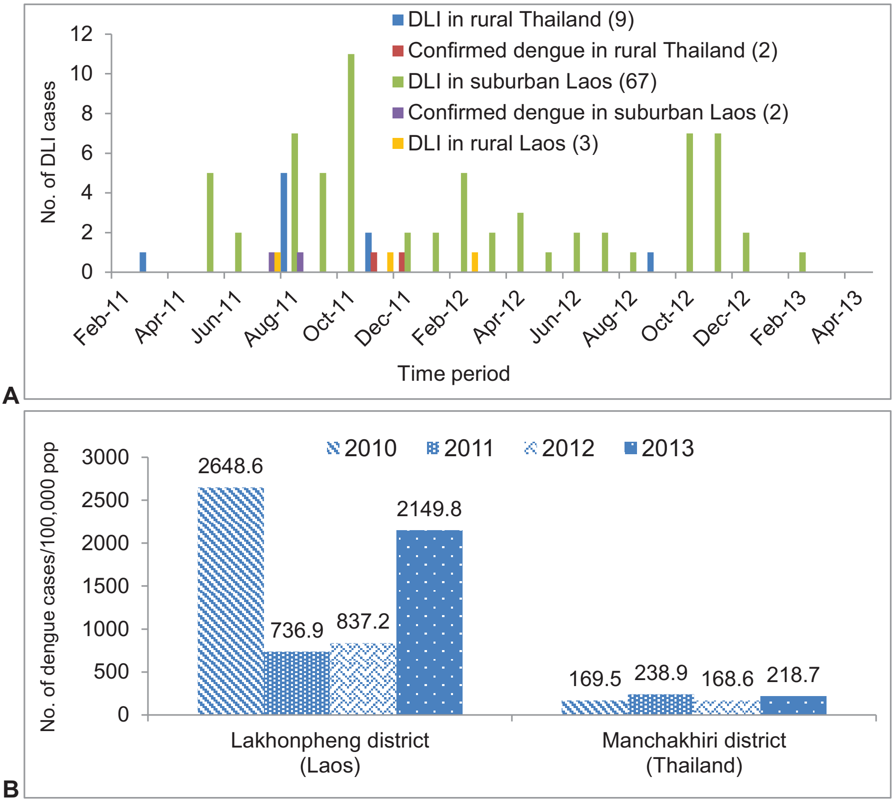
Secondary dengue data collected from the Thai national surveillance system provided by the disease surveillance unit of Manchakhiri district hospital showed that there were only five and two confirmed dengue cases in 2011 from the suburban and rural village, respectively. Of these seven cases, only one from the rural village was enrolled in our study and was also confirmed as positive of DENV infection. The other six cases were not in the selected households. In Laos, no dengue surveillance data at the village level were available from the surveillance system.

District-level secondary dengue surveillance data obtained from the national surveillance systems in Laos and Thailand showed at least a 3-fold higher dengue incidence in Lakhonpheng district (Laos) than in Manchakhiri district (Thailand) (Figure 2B). In Lakhonpheng district the incidence of dengue in 2010 was more than three times higher than in 2011 and 2012, and slightly higher than in 2013. In Manchakhiri district, dengue incidence was stable and low (<240 cases/100,000 population) during 2010-2013.

Figure 2.

A. Temporal distribution of dengue-like illness (DLI) and confirmed dengue in suburban and rural villages in Lakhonpeng district, Laos (DLI= 72) and Manchakhiri district, Thailand (DLI= 11). Figures in brackets indicate number of cases

B. Dengue incidence in Lakhonpheng district of Laos and in Manchakhiri district of Thailand (2010-2013). Figures represent the number of dengue cases per 100,000 populations reported in the districts in Laos and Thailand where the study villages are located (secondary data derived from the national surveillance system)



Risk factors and dengue-like illnesses (DLI)

The results from the univariate (Table 2) and the multivariate (Table 3) analyses were similar. The multivariate analysis showed that risk factors of DLI infection in suburban Laos were associated with age and occupation, while no evidence was found being associated with *Ae. aegypti* pupal indices (Table 3). People in the age range of 15-20 years were about five times more likely to show DLI symptoms as compared to children under five years old. People who were involved in service and "other" (retired and children) occupations had a 3-fold higher risk of DLI compared to people with agricultural occupation. In rural Thailand, the multivariate analysis showed no significant associations between DLI and any risk factor (Table 3).

Regarding dengue protection methods applied by the locals (Table 1), mosquito nets were used by 100% of the respondents in suburban and rural Laos and by 73% of respondents in rural Thailand. Other methods applied to avoid mosquito bites such as indoor insecticides spray, mosquito coils, repellents, etc. were rarely recorded in this setting.

Table 2. Univariate analyses of risk factors associated with dengue-like illnesses (DLI) in suburban Laos and rural Thailand (Odds ratio (OR) ([95% confidence intervals, CI] p-value). Numbers in bold indicate significant associations (p<0.05)

		%	Laos – Suburban village (n= 567)	%	Thailand – Rural village (n= 402)
Socio-demography					
Age group	0-5	13	1	7	1
	>5-10	10	1.6 [0.5-4.8] 0.390	5	NA
	>10-15	14	2.1 [0.8-5.5] 0.136	5	NA
	>15-20	12	**2.7 [1.0-6.9] 0.046**	8	NA
	>20-25	6	1.0 [0.3-4.1] 0.970	2	NA
	>25	45	1.3 [0.5-3.2] 0.560	73	NA
Gender	Male	48	1	50	1
	Female	52	1.1 [0.7-1.8] 0.572	50	4.6 [0.9-21.1] 0.052
Education	≤Primary school	53	1	77	1
	>Primary school	47	**2.3 [1.4-3.8] 0.001**	23	1.9 [0.6-6.5] 0.311
Occupation	Agriculture	31	1	65	1
	Service	16	**2.2 [1.0-4.8] 0.041**	3	NA
	Commerce	6	1.3 [0.4-4.8] 0.643	2	NA
	Unemployed	2	3.7 [0.8-16.6] 0.086	2	4.5 [0.6-36.8] 0.158
	Student	26	**2.6 [1.3-5.2] 0.005**	14	1.3 [0.3-6.3] 0.734
	Other*	19	1.6 [0.7-3.6] 0.229	14	0.7 [0.1-5.3] 0.694

Room occupancy rate	>2.5 persons/room	56	1	30	1
	≤2.5 persons/room	44	0.8 [0.5-1.3] 0.391	70	1.2 [0.3-4.4] 0.827
Housing material	Cement	18	1	12	1
	Cement-wood	42	1.1 [0.6-2.2] 0.737	63	NA
	Wood	40	0.9 [0.5-1.8] 0.788	25	NA
Wealth status	Poor	29	1	14	1
	Intermediate	42	1.3 [0.7-2.4] 0.375	53	0.3 [0.1-1.1] 0.065
	Rich	29	1.5 [0.8-2.8] 0.227	33	0.3 [0.1-1.5] 0.144
***Aedes aegypti* pupal indices**					
No. of pupae per household		100	1.0 [0.9-1.0] 0.917	100	NA
No. of pupae per person	0-0.49	67	1	51	1
	0.5-1.5	9	0.7 [0.2-1.9] 0.474	21	0.6 [0.1-2.6] 0.450
	>1.5	24	1.2 [0.7-2.0] 0.580	28	NA

*Other: retired and children, n: no. of individuals

Table 3. Multivariate analyses of risk factors associated with dengue-like illnesses (DLI) in suburban Laos and rural Thailand (Odds ratio (OR) ([95% confidence intervals, CI] p-value). Numbers in bold indicate significant associations (p<0.05)

		%	Laos – Suburban village (n= 567)	%	Thailand – Rural village (n= 402)
Socio-demography					
Age group	0-5	13	1	7	1
	>5-10	10	2.1 [0.6-6.9] 0.251	5	NA
	>10-15	14	3.4 [0.8-14.8] 0.097	5	NA
	>15-20	12	**4.8 [1.2-19.6] 0.029**	8	NA
	>20-25	6	2.1 [0.4-11.9] 0.406	2	NA
	>25	45	2.8 [0.8-10.5] 0.122	73	NA
Gender	Male			50	1
	Female			50	4.2 [0.9-19.3] 0.068
Occupation	Agriculture	31	1		
	Service	16	**2.4 [1.1-5.4] 0.028**		
	Commerce	6	1.4 [0.4-5.0] 0.606		
	Unemployed	2	3.2 [0.7-14.7] 0.136		
	Student	26	2.3 [0.8-6.1] 0.104		

	Other*	19	**3.5 [1.1-11.0] 0.031**		
Wealth status	Poor			14	1
	Intermediate			53	0.3 [0.1-1.2] 0.081
	Rich			33	0.3 [0.1-1.4] 0.129

*Other: retired and children, n: no. of individuals

Discussion

Dengue and dengue-like illnesses (DLI)

A total of 83 cases of DLI were recorded among 2,007 inhabitants followed during two-years in four study villages, one rural and one suburban each, in Thailand and Laos (Table 1). Most of the cases were recorded during the rainy season, which is consistent with the dengue literature.[1,4] The incidence of DLI in Laos was two times higher than in Thailand. This corresponded with the district-level dengue surveillance data reported from the same study periods in both countries (Figure 2B), where dengue incidence in Lakhonpheng district (Laos) was 3-4 times higher than in Manchakhiri district (Thailand).

There were no DLI cases in the suburban village in Thailand, although the national surveillance system reported five dengue cases from this village (from households that were not included in this study). Conversely, fewer cases of DLI were recorded in rural Laos (3 in total) compared to suburban Laos. This may have been a result of underreporting DLI cases in the rural village due to poor knowledge and little concern for health. In addition, compared to the other three study sites, rural Laos is isolated and has no functional health facility. It is also possible that the village health assistants were not motivated to collect information, or that household members were hesitant to provide information when interviewed.

Only four of the 83 DLI cases (4.8%) were confirmed as dengue in this study. Since the study was conducted in a dengue endemic area, it is possible that some of the DLI cases recorded could have been undetected dengue or other arboviral infections, such as chikungunya or Zika, which are also transmitted by dengue vectors and present similar clinical manifestations as dengue.[10] According to a recent outbreak of DLI in Pernambuco of Brazil (2015), it was found that 11.7% and 1.3% of the cases were confirmed dengue and chikungunya, respectively, with Zika accounting for 40.2%.[26] A cohort study conducted in Indonesia, Malaysia, Philippines, Thailand and Vietnam found that, among 1,500 healthy children aged 2-14 years, the most common causes of acute febrile illness (≥38°C for ≥2 days) were chikungunya, scrub typhus and dengue.[27] In 2012, chikungunya outbreaks were reported for the first time in Moonlapamok and Khong districts in Champasak province in southern Laos.[28] A year later in 2013, co-infection of chikungunya and DENV was also detected in the same province during the dengue outbreak.[9] In Thailand, a number of sporadic cases of chikungunya have been reported since its first outbreak in the country in 1958,[29] with a second outbreak occurring in Khon Kaen province in 1991.[30] The most recent chikungunya outbreak was in 2008-2009 and accounted for 52,057 cases in 58 provinces,[31] most of the cases were found in southern Thailand.[31] Co-circulation of arbovirus in these settings poses many challenges for disease diagnosis and early response to outbreaks.[32] In addition, other infections could also be responsible for the observed DLI infections in these settings, for example scrub typhus, influenza, Japanese encephalitis and leptospirosis which are common causes of acute febrile illnesses in Laos.[17,33] Leptospirosis and scrub typhus have been shown to be major causes of acute febrile illnesses in rural Thailand.[18]

Other possible reasons for the low number of confirmed dengue cases could be a result of poor sample preservation caused by intermittent power supply, that may have affected the temperature of the stored dengue samples or that the test method used was not sensitive enough. Although the method is claimed to have a sensitivity of 93%,[21] some positive samples could have been missed.

This study revealed circulation of DENV serotype 1 in Laos and serotype 2 in Thailand, a finding that corroborates each country's national surveillance data, where these serotypes were detected in the 2010 outbreak. DENV-1 serotypes were detected in Laos during 2007-2011 and accounted for the highest proportion of serotypes (38%) during the 2010 outbreak, followed by DENV-2 (30%), DENV-3 (22%) and DENV-4 (10%).[1] In Thailand, 54.6% of the DENV serotypes isolated in 2010 were DENV-2, followed by DENV-1 (25.5%), DENV-3 (15.3%) and DENV-4 (4.6%).[4]

Risk factors of dengue-like illnesses (DLI)

The risk factors significantly associated with DLI infection in suburban Laos were age and occupation. In rural Thailand, however, no risk factor was significantly associated with DLI infections. Individuals in the age group of 15-20 years were found to be associated with DLI infection which is in agreement with a previous study conducted in Brazil.[34] The large dengue outbreak that occurred in Laos in 2010 also revealed that the most affected age group was between 10-19 years.[1] In a review of dengue in Thailand, however, the highest incidence rates of dengue reported in 2000 to 2011 were between 10-14 years.[4] Similar findings were found during 2009 chikungunya outbreak in Thailand where the most affected age groups were 10-14 years.[31]

People involved in service occupations were associated with DLI infection in this study (Table 3). It was revealed that 80% had an educational level higher than high school. A previous study found that people with higher education (secondary or higher educational degrees) were significantly associated with dengue infection.[35] This may relate to travel patterns to work or job seeking outside their own residences thus increasing their chances of contracting and/or disseminating dengue infections compared to those with lower educational levels. Human movement as a result of socio-economic development is a known factor favoring the spread of dengue and other vector-borne diseases.[36-37]

Pupae per person has been proposed as a better indicator of dengue transmission than the traditional *Stegomyia* indices (House index, Container index, Breteau index) because it is a better estimate of the number of adult mosquitoes.[38-40] However, households with a high number of *Ae. aegypti* pupae per person were not associated with DLI infections (Table 3), even though the number of pupae per person found in the study villages (except rural Laos) (Table 1) were above the proposed transmission threshold models of 0.5-1.5 pupae per person.[25] This was also the case in a study conducted in the Republic of Palau, where DLI infections were not associated with the pupal index, instead the households reported with DLI were significantly more likely to be associated with potential breeding sites of mosquitoes than those without.[16] Currently, Bowman et al (2014)[40] also concluded from their systematic review on correlation between vector indices and dengue transmission that, there is little evidence of a relationship that can be utilized to predict dengue outbreaks. Further studies are therefore needed to find out more reliable indices.

Conclusion

The surveillance of DLI infections in a general population is rarely conducted in suburban and rural villages in Laos and Thailand. The occurrence of DLI was higher in Laos than in Thailand and higher than dengue in the national surveillance data of both countries. Risk factors of DLI in suburban Laos were age and occupation, but not associated with the proposed dengue transmission threshold models of 0.5-1.5 pupae per person. Among the positive cases, DENV serotype 1 and serotype 2 were detected in suburban Laos and in rural Thailand, respectively. Additionally, a hypothesis is that other febrile illnesses such as chikungunya and Zika, may be circulating in these locations. The findings presented here corroborate those from the national dengue surveillance programs, highlighting the importance of continued dengue and dengue vector surveillance, and indicating the need to expand surveillance programs to incorporate other febrile illnesses such as chikungunya and Zika, that are transmitted by *Ae. aegypti* – the same mosquito that transmits dengue. This will have significant impact on early detection of these diseases and subsequent prevention of outbreaks.

Authors' contributions

NV, HJO, TAS conceived and designed the study. NV conducted the field work, analysed and interpreted the data and drafted the manuscript. RS contributed in the study design, supervised data and water sample collection and data analyses. ND conducted the entomological survey. All authors approved the manuscript.

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Ethical approval

All participants and guardians of children signed informed consent to participate in the study. The study was approved by the National Ethics Committee for Health Research (NECHR), Ministry of Health, Vientiane, Lao PDR (No. 03/NECHR) on 17 December 2010 and by the Ethical Committee of Phramongkutklao College of Medicine, Bangkok, Thailand (S033h/53) on 21 March 2011.

Conflicts of interest

The authors declare that they have no competing interests.

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Errata

Page number	Para-graph	Change from	Change to
14	At the end	Error! Bookmark not defined. Individual pages	Individual pages **(Remove "Error! Bookmark not defined")**
15	1	Southeast Asia	South-east Asia
19	2	Southeast Asia	South-east Asia
20	3	Diarrhoea incidence	Diarrhoeal incidence
21	3	Diarrhoea disease	Diarrhoeal disease
25	1	The components A, B and C in the first upper boxes	The components A, B and C in the boxes on the left
42	3	Diarrhoea incidence	Diarrhoeal incidence
43	3	A. Socio-demographic factors on DLI	A. Effect of socio-demographic factors on DLI
2 of paper 1	5	**128** households in the suburban and rural villages in Laos, and from a total of **139** and **272** households	**130** households in the suburban and rural villages in Laos, and from a total of **272** and **139** households
The title of attached paper 2	1	Stenström TA, Dada N, Overgaard HJ	Stenström TA, Dada N, Overgaard HJ **(Remove the hyperlink from these author names)**
3 of paper 3	1	Diarrhoea disease	Diarrhoeal disease
3 of paper 3	2	Diarrhoea disease	Diarrhoeal disease
4 of paper 3		Study Design	Study design
6 of paper 3	2	(Vyas *et al.* 2006)	(Vyas and Kumaranayake 2006)
9 of paper 3	1	Diarrhoea disease	Diarrhoeal disease
10 of paper 3	3	100,000 populations than Laos.	100,000 populations than Laos **(Figure 4)**.
11 of paper 3	1	Laos and Thailand (2010-2013).	Laos and Thailand **(2011-2013)**.
19 of paper 3	4	after an intervention diarrhoeal intervention.	after diarrhoeal intervention.
22 of paper 3	Reference no. 4	Brown J. M., Proum S. & Sobsey M. D.	Brown JM, Proum S, Sobsey MD.
23 of paper 3	In reference list	No reference in the list	Pradhipasen M, Chareonkul C, Nitnara S, Taweedej J, Pamonprawat A. The underreporting of childhood diarrhoea in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1997, 28(2), 391-394. **(This reference should come after Platts-Mills JA et al 2015)**
12 of paper 4	2	Six times	Five times