Risk and protective factors for primary and secondary dengue infections among school-aged children in the Meuang District of Rayong Province, Thailand

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By

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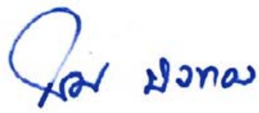

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Abbreviations

DEET	*N*,*N*-Diethyl-*meta*-toluamide
DEN-1	Dengue virus type 1
DEN-2	Dengue virus type 2
DEN-3	Dengue virus type 3
DEN-4	Dengue virus type 4
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
ELISA	enzyme-linked immunosorbent assay
IgG	Immunoglobulin type G
IgM	Immunoglobulin type M
JE	Japanese Encephalitis
NT	Neutralization Test
OR	Odd ratio
PRNT	Plaque Reduction Neutralization Test
SDNT	Single-Dilution Plaque Reduction Neutralization Test
WHO	World Health Organization

Abstract

Background: Dengue is a major public health problem in Thailand. Many risk factors of dengue infection were studied but they rarely explored factors associated with primary and secondary infections. This study aimed to determine risk and protective factors in different type of dengue infection.

Methods: A matched case-control study was conducted. A case was identified as either primary or secondary dengue infection by single-dilution plaque reduction neutralization testing. A control was matched with the case by age and school location. Behavioural information after birth was obtained from caregivers. Conditional logistic regression analyses were applied to adjust for confounding factors among significant determinants.

Results: Seventy eight cases including 40 primary and 38 secondary infections were recruited. Significant risk factors of primary infection included a child born in an urban area ($p = 0.03$) and no migration from the home town ($p = 0.03$). The protective factor of secondary infection was the child had a parent as a caregiver at preschool age ($p = 0.03$) and sleeping under bed netting during weekend at home in school age ($p = 0.03$). The risk factor of secondary infection was that the child had a fever very often (≥ 3 times per year by average) ($p = 0.03$) and the child never changed schools in school age ($p = 0.004$).

Conclusions: Our study confirmed that different determinants played a role in primary and secondary dengue infections. Interventions for dengue prevention should specifically be designed to target children at each age-groups.

Nr of words: 245

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Background information of Thailand and Rayong Province

Thailand is a tropical country situated at the centre of the Indochina peninsula in Southeast Asia. The country shares a border with Myanmar to the west, Laos and Cambodia to the east and Malaysia to the south. The area covers 513,115 km² (198,114 sq. mi.) with a densely populated central plain, a north-eastern plateau, a western mountain range and a southern coastal land mass. There are three seasons; namely, summer (February-April), a rainy season (May-October), and winter (November-January). The population in 2010, which was surveyed by the National Statistical Office, the Ministry of Information and Communication Technology of Thailand, was approximately 66 million, of which the number of children below 15 years of age was about 12.7 million (19.2%); the working-age population (15-64 years old) was 44.8 million (67.9%); and the elderly ($\geq$65 years old) was 8.5 million (12.9%).[1] The median age of the population was 34.7 years with the country steadily moving to be a population ageing society. In 2010, the crude birth rate was 13.01 per 1,000 persons and the life expectancies of Thai males and females were 70.4 and 77.5 years, respectively.[2] Of the population aged 15 years or over, 46.5% had higher than elementary school education.[2]

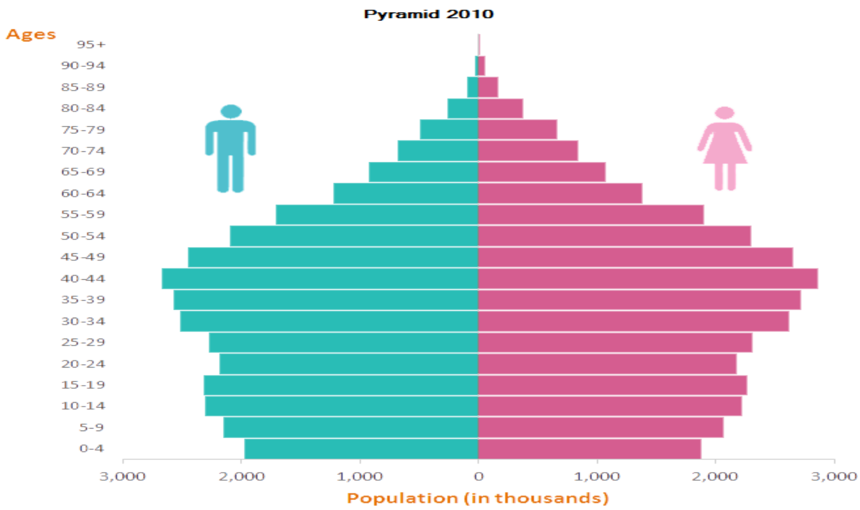

Figure 1 Population structure of Thai citizen divided by age-groups and gender, 2010

Source: The National Statistical Office, Ministry of Information and Communication Technology, Thailand

Thailand is an agricultural country and the main products are rice, cassava (tapioca), rubber, corn, sugarcane, coconuts and soybeans. However, industrial manufactures has grown rapidly in the last two decades and the tourism business is a significant source of the country's income. Thailand is graded as an upper middle income country. In 2013, the gross domestic product per capita in Thailand was 3,437.84 US dollars as estimated by the World Bank.[3] Health infrastructure has rapidly been developed with at least one public hospital in each of the 878 districts nationwide. In 2010, a universal health insurance scheme, the Universal Coverage Scheme, provided by the Thai government covered approximately 47.7 million of the total population (75.7%) under the National Health Security Office.[4]

Road transportation is the main mode of transportation between provinces while the numbers of airline passengers on internal flights have been increasing in the recent years. Most households use tap water as a source of water supply (81.2%), followed by water from wells (7.5%), underground water (7.3%), watercourses (1.8%) and rain water (1.4%).[1]

Rayong Province is one of the 77 provinces of Thailand and it is located about 220 km to the south-east of Bangkok along the east coast of the Gulf of Thailand. Most of the area of Rayong Province is mountainous with interspersed flat plains, cultivated for fruit plantations and forests, and there are many beaches along the coastline.

Rayong Province is among the top 5 provinces in terms of economic and social development. There have been a huge number of internal migrations, as well as an influx of migrant workers from neighbouring countries to work in many factories. The Department of Provincial Administration of Ministry of Interior estimated that 1.1 million people lived in the 8 districts of Rayong Province in 2010.[5] Of those, about 55% were registered as residents of the province while the remainder was mostly migrant workers.

The province has a good road system and many sea ports for agricultural and industrial product transportation, as well as for tourism. There are 8 major industrial estates in Rayong Province, and these produce a large demand for skilled and unskilled workers.

The Meuang District of Rayong Province is the provincial capital and is a cosmopolitan mixture of Thai residents, Thai migrants and foreign migrants from neighbouring countries. It includes 15 sub-districts and 83 villages in the district.[6] Differences in characteristics of communities vary greatly from rural to urban and within the industrial estate areas in this unique district. Residents of the Meuang District have access to medical services at health promotion hospitals located in every sub-district.

Figure 2 Map of Thailand and Rayong

Problem statement

Globally, dengue disease is a main vector-borne disease in tropical countries. The disease is transmitted by *Aedes* mosquitoes. Bhatt S. et al. estimated that 390 million people were infected with dengue infection in 2010, of which 96 million cases (24.62%) became symptomatic dengue diseases and, of which approximately 66.8 million cases (69.58%) occurred in Asia.[7] Three classifications of clinical characteristics after dengue viruses infection, including dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), have been used for reporting criteria in Thailand. Dengue virus consists of four closely related, but genetically distinct serotypes.[8] The four dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) have been circulating in the country for over five decades.[9] In Thailand, DHF was first reported in 1958[10], and the disease has mainly occurred in children below 15 years of age with the highest morbidity in 1987 (325/100,000 population). However, there has recently been a shift in the age distribution towards older individuals in the last two decades. Young school age children at 5-14 years had the highest attack rate of dengue infection in 1990s [11].

Justification

Infection by one dengue serotype confers lifelong immunity against infection by that same serotype, but pre-existing immunity appears to enhance the severity of subsequent heterologous infections.[8,12] The spectrum of disease ranges from asymptomatic and mild febrile illness in the majority of infections to severe haemorrhagic fever in a varying proportion of cases [13,14,15]. Even though secondary infection is considered to be the main risk factor for severe disease, the roles of other factors such as age, sequence of infecting serotypes and viral factors are not clear[13]. The association between age, risk of infection and clinical disease has been particularly elusive; in many hyperendemic settings, severe dengue occurs almost exclusively in children, but this age range coincides with that of secondary infections.

Some risk factors for dengue infection have been well demonstrated in both non-epidemic and epidemic circumstances. The risks include sex [16, 17, 18], increasing age [16, 17, 18, 19, 20, 21] deprived socio-economic status [16, 17] and an environment prone for vector infestation [16, 17, 19, 20, 21, 22, 23]. However, most studies have been based on clinically identified cases, or if based on seroprevalence survey, they have used enzyme-linked immunosorbent assay (ELISA).[16, 17, 18, 19, 20, 21, 22, 23] The dengue ELISA either Ig M antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) or IgG enzyme-linked immunosorbent assay (IgG-ELISA) techniques are usually interfered with by other flaviviruses in the given area of study such as Japanese encephalitis (JE) virus in Asia and yellow fever virus in Latin America.[24] Because of the decreased specificity of these assays, estimates of seroprevalence will tend to be higher and mis-specify the serological status of individuals in many cases. The best method for serological detection of dengue immunity is plaque reduction neutralization testing such as the Plaque Reduction Neutralization Test (PRNT) or the Single-Dilution Plaque Reduction Neutralization Test (SDNT), both of which have higher specificities to each serotype of dengue virus. These tests can differentiate immunity derived from dengue exposure, dengue viruses from other flaviviruses and individuals experiencing primary infection from those having experienced secondary infection in the past.[25] Despite the increased sensitivity and specificity of the Neutralization Test (NT), a limited number of previous studies have used NT for serosurveys. In this study, we utilize the NT assay to minimize the impact of cross-reactivity with other flaviviruses. We also use NT assays for JE antibody to identify potential false positive results from cross-reactivity with JE due to the high level of JE immunity among children in our serosurvey. This high level of immunity has been caused by the universal coverage of JE vaccine introduced in 1997 and the high burden of JE disease [26].

The outcomes of previous studies [16, 17, 18, 19, 20, 21, 22, 23] were mainly focused on environmental and socioeconomic risk factors. There has been more limited work on behavioural risks such as daily activities, caretaking, traveling, migrating, assigned sleeping in daytime for kindergarten and etc. The risk of first time infection (primary infection) might be affected by caretaking conditions, migration, early or delayed attendance of nursery, playing time in daytime and number of peers. The secondary infection risks might be related with older-aged children's activities including migration for an extended period of time or school-based activities. Normally, the child who had been infected was assumed to have the same serotype that had been circulating in the community at that time. Thus, the possible risk factors of secondary infection may be different from primary infection such as the migration of a child to a new setting which has a different type of dengue circulation if young and old children move in and between communities at greater rates. Living conditions such as home type and characteristics of the local environment such as mosquito densities in temples, markets, school and factory, which are potential sources of vector exposure, may play a role in both primary and secondary infection. Thus, the risk factors of unclassified dengue infection (the presence or absence of dengue immunity) in previous reports by ELISA may not be appropriate to infer conclusions for all age groups of children because of differences in exposure with age. The classified types of infection (primary and secondary) by age and matched in the same age group will help identify risk factors important for each stage of life and help policy makers pinpoint disease control. By identifying risk factors associated with specific age groups, public health authorities can implement prevention strategies and policies that target the real risks that each age group experiences. Risk factors for secondary infection are important to the clinician because these are risks are associated with severe and fatal outcomes. Identification of these risk factors will help the clinician and public health officer to target resources to minimize severe clinical outcomes.

Objectives

Overall objective:

To determine the risk and protective factors of dengue immunity and prior dengue infection among school-aged children (5-18 years old) in the Meuang District of Rayong Province, Thailand in 2011.

Specific objectives:

Specific objective 1: Determine the risk and protective factors of primary dengue infection by comparing characteristics of those having evidence of past primary infection with those having no evidence of past infection in the same age group and school location among school-aged children of 5-18 years old living in the Meuang District of Rayong Province, Thailand.

Specific objective 2: Determine the risk and protective factors of secondary dengue infection by comparing characteristics of those having evidence of past exposure to two or multiple infections of dengue with those having no evidence of past infection in the same age group and school location among school-aged children of 5-18 years old living in the Meuang District of Rayong Province, Thailand.

Specific objective 3: Determine the risk and protective factors of total dengue infection by comparing characteristics of those having evidence of past exposure with those having no evidence of past infection in the same age group and school location among school-aged children of 5-18 years old living in the Meuang District of Rayong Province, Thailand.

Methods

Study design

This is a matched case-control study, using primary data from interviews of students and/or their guardians/caretakers. The study is based on and also uses secondary data of laboratory results from a previous serosurvey by SDNT among 1,811 school-aged children conducted in the Meuang District of Rayong Province [27].

Study setting

Rayong Province, located in South-eastern Thailand, is hyperendemic for dengue and has historically has been reported to have one of the highest numbers of cases in Thailand. In 2010, the incidence of dengue diseases was 202.9/ 100,000 persons, and of these, 111.7/100,000 persons were cases of DHF. This study was conducted in the Mueang District of Rayong Province. It has a total area of 514.5km^2 and a population of 229,657 (as surveyed in 2008) [28]. There are 68 schools in the district. The Thai school system consists of 14 basic years (2 pre-primary, 6 primary levels and 6 secondary levels). It is estimated that over 95% of children attend primary schools to complete grade 6 or higher.

Study approval

The study was reviewed and approved by the Research Ethics Committee of the Institute for the Development of Human Research Protections (IDHRP), the Ministry of Public Health, Thailand on May 10th 2011 and the Royal Tropical Institute Amsterdam, the Netherlands on August 10th, 2011.

Study participants

The secondary data used as starting point for the current study, involved school children aged 5-18 from the Mueang District of Rayong Province who had participated in a previous serosurvey of dengue infection by SDNT in 2010 [28]. The 2010 study randomly recruited 28 schools among 96 schools in the Mueang District of Rayong Province with probability proportional to the number of students. Class and classrooms were randomly sampled with a sampling frame proportional to the number of students in each of them. Overall, 1,811 students participated in the 2010 study. There are laboratory results for dengue and JE antibodies by SDNT for all these study participants.

For the primary collection phase of our study, we recruited 93 cases by simple randomization from the original list of participants who had lab results of primary or secondary dengue infection. We were only able to find suitable controls for 78 cases. Because of limited number of controls available to match with the randomized cases in the same class and school, we eventually had 40 cases of primary dengue infection and 38 cases of secondary dengue infection to be included in the analysis.

Inclusion Criteria

Subjects were chosen from the 1,811 students in the 2010 study in Rayong Province who had laboratory results of both dengue and JE virus immunities.

Case definition: a student aged 5-18 years old who has evidence of a prior primary or secondary dengue infection by SDNT

An individual with primary infection is defined as a participant who has a detectable dengue neutralization titre (>10) for a single type of dengue serotype by SDNT and an undetectable titre for all other serotypes.

An individual with secondary infection is defined as a participant who has a detectable titre (>10) to two or more serotypes of dengue by SDNT.

Control definition: a student aged 5-18 years old who is found to have an undetectable dengue neutralization titre (<10) for all dengue serotypes by SDNT

Control selection: Controls were selected among children who had no detectable dengue neutralization titre, and these controls were matched with cases by the following criteria.

1. A control must have an age closest with the case among all potential controls, the difference in age must not be more than 12 months and educational level must be in the same category with categories defined as follows:
   1) Kindergarten

2) Lower primary school (grades 1-3)
3) Upper primary school (grades 4-6)
4) Lower secondary school or middle school (grades 7-9)
5) High school or technical college (grades 10-12)

2. A control must study in the same school or same district as the case. If no control exists to meet criteria 1 (above) in the same school or same district, the nearest school or district will be selected.

Justification for matching on age

A shift in the age of dengue cases is being observed in Thailand even though the number of cases remains fairly constant. This has potentially large implications for clinical practice and public health. The shift could reflect a decrease in the hazard of infection, but changes in the surveillance patterns and/or in the virus itself could potentially have generated a similar phenomenon. Insight into the nature of the shift and of the factors that might be driving it is fundamental for adequate planning of future control interventions. Figure 3 shows the increasing mean age among dengue cases reported over the past 50 years in longitudinal passive surveillance data from the Bureau of Epidemiology of the Ministry of Public Health, Thailand.

Age is associated with immune status as an increase in years of age is correlated with a higher positive rate of dengue immunity. Therefore, the selected cases and controls are matched by age to adjust for the impact of different lengths of time at risk across individuals with different ages in this study.

Justification for not matching on sex

We did not match by sex because of no significant difference in observed incidence rates of dengue between genders in Thailand. In addition, we had a limited number of potential controls for age-sex matching in the study pool.

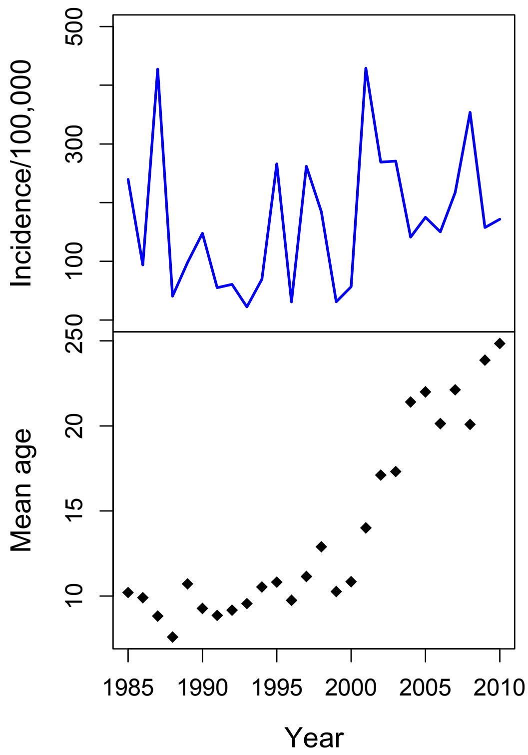

Figure 3 Dengue incidence and mean age of dengue cases in Rayong 1985-2010

Source: the Bureau of Epidemiology, Ministry of Public Health Thailand

Exclusion Criteria

Students included in the survey from 2010 who had emigrated from the Meuang District of Rayong Province were excluded**.**

Case-control ratio: 1:1 in case with primary and secondary immunity.

Study questionnaire

Three major categories of personal information were obtained by interview (a total of 64 questions). The information includes as follows:

1) Personal information (7 items) included age, gender, ethnicity, birth place, birth weight, vaccination history and history of JE vaccination.

2) Behavioural information

2.1 Infantile age (< 1 year old, 7 items) included the infant's care taker, the infant's habitat, the type of residence, migration to another place for an extended period of time (> 1 month period), history of febrile illness, history of doctor's visits and history of dengue disease diagnosed by a health professional.

2.2 Preschool age (1 – 5 years old, 18 items) included the child's care taker, the child's habitat, residence characteristics, migration to another place for an extended period of time (> 1 month period), history of febrile illness, history of doctor's visits, history of dengue disease diagnosed by a health professional, nursery attendance, age of first time nursery attendance, travel to another province, indoor sleeping during the daytime, sleeping under bed netting during the daytime, sleeping under an electric fan, showering before sleeping, the number of peers, the time spent indoors during the daytime, the frequency of having lunch outside the home and the time of dinner.

2.3 School age (6 – 18 years old, 17 items) included the child's care taker, the child's habitat, residence characteristics, migration to another place for an extended period of time (> 1 month period), history of febrile illness, history of doctor's visits, history of dengue disease diagnosed by a health professional, travel to another province, sleeping under an electric fan, showering before sleeping, the number of peers, the time spent indoors during the daytime, the frequency of having lunch outside the home, the time of dinner, the frequency of changes of school, the frequency of attending tutorial classes in the evening and history of classmates diagnosed with dengue disease.

3) Household information (15 items) included the duration of stay of the child in his or her current habitat, habitat characteristics, the dimensions of the common area in the child's habitat, having domestic animal or pet, having outdoor surrounding vegetation, having indoor plants, the type of water supply, having a shower in a bathroom, having a toilet, having bed netting, having unused tyres in the home area, having discarded containers in the home area, experience of mosquito bites in the home area and having mosquito larvae in the home area.

Single-dilution plaque reduction neutralization testing

A detailed description of the SDNT assay used is available in the previous study that has been mentioned [28]. In brief, serum samples were considered positive for dengue neutralizing antibodies if they neutralized >70% of plaques at a single 1:10 dilution. This dilution/neutralization level has been shown to be optimal for differentiating people who have been exposed to dengue from those who are immunologically naïve, but this level is suboptimal for classifying homotypic vs. heterotypic immunity.

Statistical analyses

The data were analysed using Epi Info software version 3.5.1 (August 13, 2008). The software was developed by the US CDC, Atlanta. We estimated the strength of association between selected determinants (either risk or protective factors) and exposure to dengue infection (both primary and secondary infection) by matched odds ratio (OR) and adjusted ORs which were tested by the Mantel-Haenzel matched-pairs analysis. Conditional logistic regression analyses were applied to adjust for confounding factors among significant determinants.

Results

Characteristics of the participants in the 2010 dengue serosurvey study

A total of 1,811 children from 90 classes in 25 schools participated in the 2010 dengue serosurvey study. Three additional selected schools refused to participate. Enrolled schools were located in 9/15 of the sub-districts within the Mueang District of Rayong Province. These enrolled schools represented urban, rural and industrial areas. Figure 4 shows the locations of the enrolled schools.

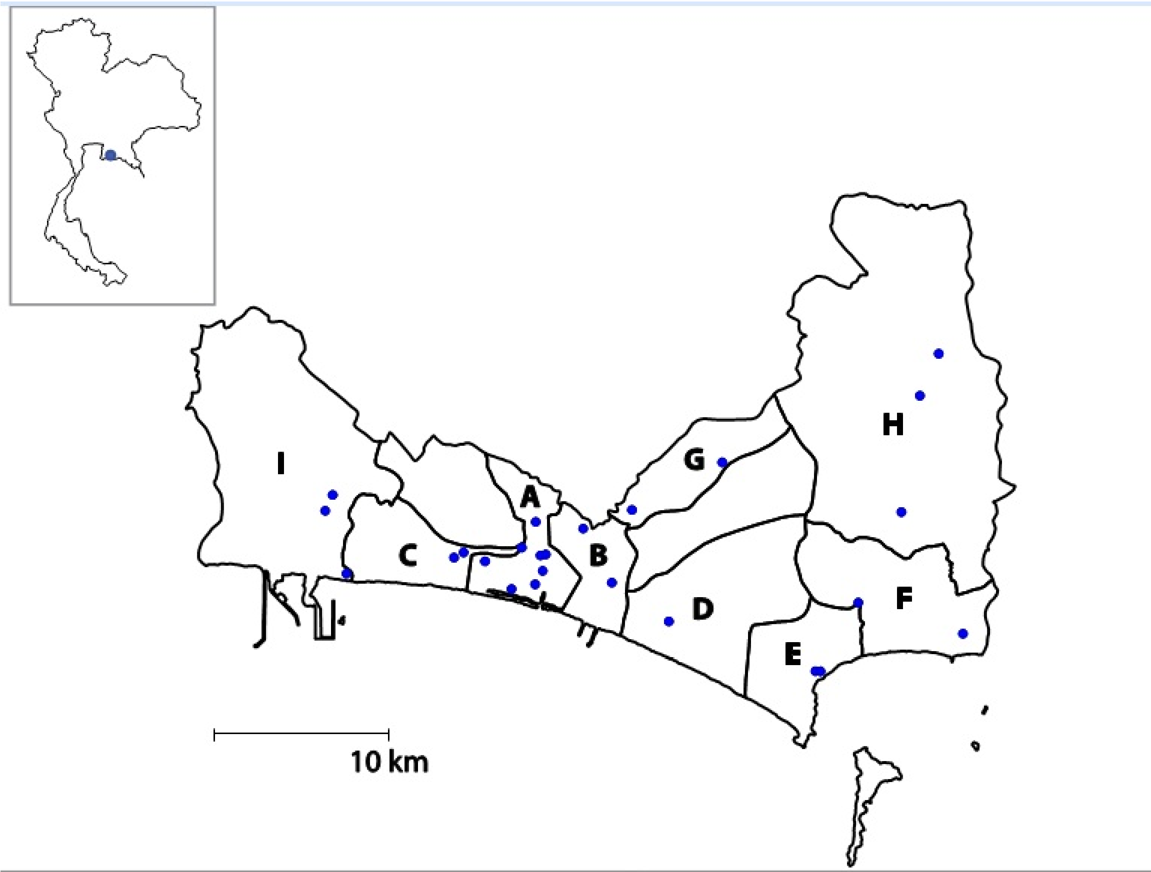

Figure 4 Map of Rayong showing location of schools, areas, and area of original study (A to H represented by sub-district)

Table 1 summarizes the characteristics of participants. The majority of children (n=1016, 56%) were enrolled in urban schools. The proportions of females enrolled were higher in rural and industrial area schools (57% and 57%, respectively) than in urban schools (51%). Reported access to electricity was universal (> 99% of the enrolled children reported having electricity at home), but only 82% of children reported having tap water at home. The reported proportions having access to tap water were significantly lower in children enrolled from rural and industrial area schools (72% and 75%, respectively) than in children enrolled in urban schools (88%). Children enrolled in the single industrial sub-district reported having lived in that particular location 2.8 years less than those from urban sub-districts, and this was significant even after adjusting for age.

Table 1 Characteristics of study participants of dengue serosurvey in 2010

	Urban *(A,B,C,F)*	**Rural** *(D,E,G,H)*	**Industrial** *(I)*	**All**
No. Children	1016	564	228	1808
No. Schools	16	9	3	25
No. Sub-districts	4	5	1	9
Mean age (se)	13.1 (.11)	11.7 (.15)	10.8 (.23)	12.3 (.09)
Female% (n)	51 (522)	57 (324)	57 (129)	54 (975)
Years in location (se)	9.5 (.2)	8.1(.2)	6.7 (.3)	8.7(.2)
Household Characteristics				
Median no. of members (range)	4 (1-11)	4 (1-10)	4 (2-10)	4 (1-11)
Electricity% (n)	100 (1016)	99 (560)	100 (228)	99.8(1804)
Tap Water%(n)	88 (896)	72 (409)	75 (171)	82 (1476)
Auto/motorbike% (n)	97 (989)	96 (543)	96 (218)	97 (1750)

3 children excluded from analysis because of insufficient/damaged sample

Prior exposure to dengue virus

Overall, 69% of the sampled children showed evidence of prior exposure to dengue virus. Almost half (46%) of the samples showed immunological evidence of exposure to JE virus.

Figure 5 shows the age-specific seroprevalence to dengue virus in the 2010 serosurvey. Seventy four percent (95% CI; 61%-87%) of the population had been exposed to dengue by age 11 years. By age 18 years, 16% (CI 95%; 0%-32%) of the population remained susceptible to dengue virus.

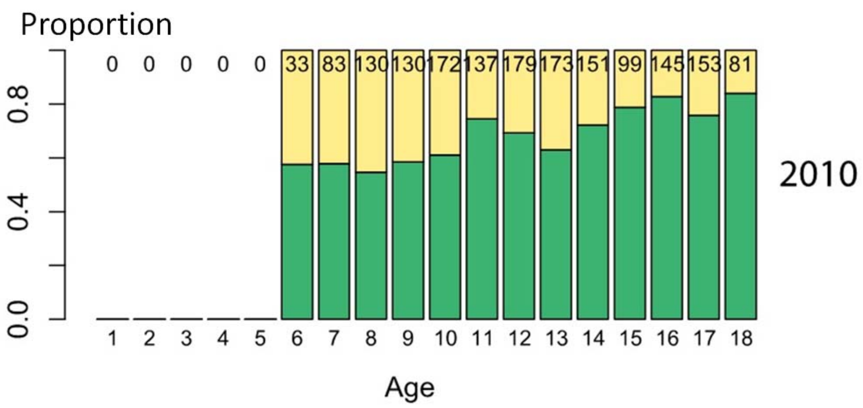

Figure 5 Age specific seroprevalence according to 2010 dengue serosurveys. (Yellow represents naïve for dengue infection and green represents dengue exposure either primary or secondary)

Among 197 participants with detectable neutralization titre to only one serotype (and no others), dengue type 3 was the most common (40.61%), followed by type 2 (22.84%), type 1 (19.80%) and type 4 (16.75%).

Characteristics of cases of primary and secondary infections

During the dengue serosurvey of 2010, a total of 78 cases of dengue infection were recruited with 40 participants having results compatible with a primary infection and 38 participants having results compatible with a secondary infection. Table 2 shows the distribution of cases by class category and figure 4 shows the gender distribution between primary and secondary immunity.

Table 2 Number of cases by class category and type of infection

Class category (N=78)	**Primary**	**Secondary**	**Total (%)**
Kindergarten 5-6 years old	6	6	12 (15.38)
Lower primary school (grade 1-3)	16	12	28 (35.90)
Upper primary school (grade 4-6)	9	15	24 (30.77)
Middle school (grade 7-9)	9	5	14 (19.95)
High school or technical college (grade 10-12)	0	0	0
Total	40	38	78 (100)

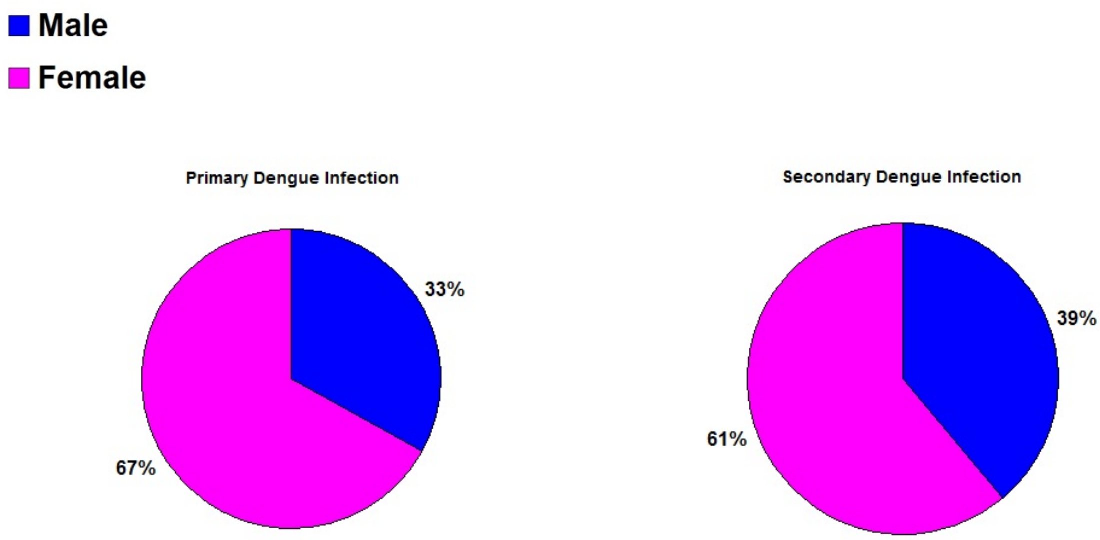

Figure 6 Distribution of genders in primary and secondary dengue infections

Most of the participants were born in the Meuang District (70.51%), followed by outside Rayong Province (25.64%) (Table 3 and figure 5). There was no difference in birth place between the primary and secondary immunity groups. Two participants were offspring of immigrant worker. One was Cambodian and the other was from Myanmar. Eighty six percent of birth weight statuses were normal (normal range: 2,500 - 3,900 grams). The proportion of participants vaccinated with JE vaccine was 64.10% (completion of 3 dosages, 60.25% and incompletion of 3 dosages, 3.85%), and the remainder had unknown vaccination statuses.

Table 3 Birth place of the participants by type of infection

Birth Place	Primary infection	Secondary infection	All
Rayong			
• Meuang district	29	26	55
• Other district	0	1	1
Outside Rayong	11	9	20
Outside Country	0	2*	2
Total	40	38	78

* Myanmar and Cambodia

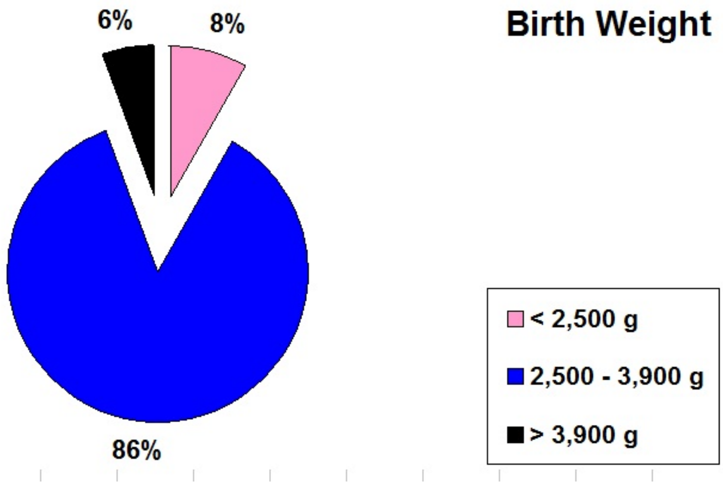

Figure 7 Birth weight of the participant (N=78)

Risk and protective factor of primary dengue infection compared to naïve participants

Table 4 demonstrates that only two significant risk factors of primary dengue infection were identified from the analysis. The risk factors were found only during the period of infantile age (after birth to 12 months years old). The risk factors of the infantile age group child are birth in an urban area defined as a large city or city or town (p = 0.03) and no migration from the home town (p = 0.03). By conditional logical regression analysis, only an urban place of birth remained a statistically significant risk factor (p = 0.04) while the no migration from the home town became nearly significant (p=0.06) (Table 5). There was no statistically significant association between primary dengue infection and determinants in older age-groups (preschool age and school age). Overall, household determinants were not associated with primary dengue infection.

Table 4 The determinants of primary dengue infection for school-aged children in the Meuang District of Rayong Province, Thailand

Factor	Unexposed case – exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
Behavioural information				
Period 1: INFANTILE AGE (After birth to 1 year of age)				
• During infancy, a parent took care of the child.	4	10	0.40 (0.12, 1.28)	0.11
• During infancy, the child was staying at the current place.	8	9	0.89 (0.34, 2.30)	0.80
• During infancy, the child's habitat was located in an urban area (large city/city/town).	9	2	4.50 (0.97, 20.83)	0.03
• During infancy, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	9	2	4.50 (0.97, 20.83)	0.03
• During infancy, the child had a fever very often (> 10 times in a year).	5	1	5.00 (0.58, 42.80)	0.10
• During infancy, the child visited to hospital/clinic often (≥ 6 times/year) due to illness.	5	3	1.67 (0.40, 6.97)	0.47
• During infancy, the child had been diagnosed with any dengue disease by a medical doctor.	1	1	1.00 (0.06, 15.99)	1.00
Period 2 : PRESCHOOL AGE (1- 5 years old)				
• During 1-5 years old, a parent took care of the child.	2	5	0.40 (0.08, 2.06)	0.25
• During 1-5 years old, the child was staying at the current place.	6	6	1.00 (0.32, 3.10)	1.00
• During 1-5 years old, the child's habitat was located in urban area. large city/city/town).	4	4	1.00 (0.25, 4.00)	1.00
• During 1-5 years old, the child never migrated from his/her home town.	8	6	1.33 (0.62, 2.84)	0.59
• During 1-5 years old, the child had a fever very often (≥ 3 times/year).	7	7	1.00 (0.35, 2.98)	1.00
• During 1-5 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	10	4	2.50 (0.78, 7.97)	0.11
• During 1-5 years old, the child had been diagnosed with any dengue disease by a medical doctor.	1	3	0.33 (0.03, 3.20)	0.32
• During 1-5 years old, the child attended nursery or kindergarten.	1	3	0.33 (0.03, 3.20)	0.32
• During 1-5 years old, the child	14	7	2.00 (0.81, 4.95)	0.12

Factor	Unexposed case – exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
attended the nursery or kindergarten in early age (≤ 3 years of age).				
• During 1-5 years old, the child never travelled to another province.	17	8	2.12 (0.93, 4.92)	0.07
• During 1-5 years old, in the daytime at home, the child often slept indoors.	9	14	0.64 (0.28, 1.48)	0.30
• During 1-5 years old, in the daytime at home, the child often slept under bed netting.	7	11	0.64 (0.25, 1.64)	0.34
• During 1-5 years old, in the daytime at home, the child often slept under an electric fan.	3	7	0.43 (0.11, 1.66)	0.20
• During 1-5 years old, in the daytime at home, the child often took a bath/shower before sleep.	7	9	0.78 (0.29, 2.13)	0.61
• During 1-5 years old, the child had ≥ 6 peers/closest friends in his/her community	5	5	1.00 (0.29, 3.72)	1.00
• During 1-5 years old, the child spent > 6 hours per day indoors at home during the daytime.	8	6	1.33 (0.46, 3.84)	0.59
• During 1-5 years old, the child often had lunch at restaurant/outside the home.	5	12	0.60 (0.22, 1.65)	0.32
• During 1-5 years old, the child usually had dinner before 6 pm.	8	9	0.89 (0.34, 2.30)	0.80
Period 3: SCHOOL AGE 6-18 years old				
• During 6-18 years old, parent was taking care of the child.	2	5	0.40 (0.08, 2.06)	0.25
• During 6-18 years old, the child was staying at the current place.	2	1	2.00 (0.18, 22.06)	0.56
• During 6-18 years old, the child's habitat was located in an urban area (large city/city/town).	3	2	1.50 (0.25, 8.98)	0.65
• During 6-18 years old, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	9	4	2.25 (0.69, 7.31)	0.17
• During 6-18 years old, the child had fever very often (≥ 3 times/year).	7	6	1.17 (0.39, 3.47)	0.78
• During 6-18 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	6	4	1.50 (0.42, 5.32)	0.52
• During 6-18 years old, the child	1	2	0.50	0.56

Factor	Unexposed case – exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
had been diagnosed with any dengue disease by a medical doctor.			(0.04, 5.51)	
• During 6-18 years old, the child never travelled to another province.	15	8	1.87 (0.80, 4.42)	0.14
• During 6-18 years old, at weekend at home, the child often slept under an electric fan.	4	7	0.57 (0.17, 1.95)	0.36
• During 6-18 years old, at weekend at home, the child often slept under bed netting.	4	11	0.36 (0.11, 1.14)	0.07
• During 6-18 years old, at weekend at home, the child often took a bath/shower before sleep.	8	11	0.72 (0.29, 1.81)	0.49
• During 6-18 years old, the child had > 6 peers/closest friends in his/her community	6	4	1.50 (0.42, 5.32)	0.53
• During 6-18 years old, the child spent > 6 hours per day indoors at home during the daytime.	8	8	1.00 (0.38, 2.66)	1.00
• During 6-18 years old, the child usually had dinner before 6 pm.	6	4	1.50 (0.42, 5.32)	0.53
• During 6-18 years old, the child never changed schools.	6	0	undefined	
• During 6-18 years old, the child took a tutorial class in the evening ≥ 3 times/week.	9	4	2.25 (0.69, 7.31)	0.16
• During 6-18 years old, the child had a classmate diagnosed with a dengue disease by a medical doctor.	0	1	undefined	
Household information				
• The child lived with the care giver in the same place for more than 5 years.	4	6	0.67 (0.19, 2.36)	0.52
• The habitat was apartment/townhouse.	11	6	1.83 (0.68, 4.96)	0.20
• The common area in village such as temple, school, grocery store or convenience store was < 1 km distance from the child's habitat	7	10	0.70 (0.27, 1.84)	0.47
• Having domestic animal/pet	9	8	1.12 (0.43, 2.91)	0.80
• Having outdoor surrounding vegetation	1	5	0.20 (0.02,1.71)	0.10
• Having indoor plants	6	3	2.00 (0.50, 8.00)	0.32

Factor	Unexposed case – exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
• Having tap water as a water supply	4	8	0.50 (0.15, 1.66)	0.25
• Having a shower in the child's bathroom	6	6	1.00 (0.30, 3.25)	1.00
• Having a water container in the child's bathroom	7	0	undefined	
• Having a toilet in the child's home	2	0	undefined	
• Child always uses bed netting.	10	4	2.50 (0.78, 7.97)	0.11
• Having unused tyres in the child's home area	9	5	1.80 (0.60, 5.37)	0.28
• Having discarded cans/ plastic/utensils in the child's home area	8	6	1.33 (0.46, 3.84)	0.65
• Having experience of mosquito bites during the daytime	3	2	1.50 (0.25, 8.98)	0.65
• Having mosquito larvae in the child's home	8	6	1.33 (0.46, 3.84)	0.59
Personal information				
• Male gender	8	13	0.62 (0.26, 1.48)	0.28

Table 5 Conditional logistic regression analysis of risk factors for primary dengue infection in school-aged children at the Meuang District of Rayong Province, Thailand

Factor	Adjusted OR	95% CI.		P-value
The infant's habitat location was urban.	<u>15.52</u>	<u>1.16</u>	<u>207.11</u>	<u>0.04</u>
The infant never migrated from the home town (defined as staying at least 1 month in a new place).	8.02	0.90	71.10	0.06

Risk and protective factors of secondary dengue infection compared to naïve participants

The determinants of secondary dengue infection were found in the period of preschool age (1 – 5 years old) and school age (6 – 18 years old). Two factors were significant during the preschool age period. The protective factor was that the child had a parent as a care taker ($p = 0.03$), and the risk factor was that the child had a fever very often (≥ 3 times per year by average) ($p = 0.03$). At school age, the protective factor was that the child often slept under bed netting during the weekend at home ($p = 0.03$), and the risk factor was that the child never changed schools ($p = 0.004$). There were no statistically significant determinants found during the period of infantile age, or relating to the household characteristics.

Among the 4 significant determinants found, only the child had fever very often (≥ 3 times per year by average) was the strongest risk factor, but it was not significant. Furthermore, at school age, the child never having changed schools and the child having often slept under bed netting at weekend are concordant with univariate results of association. However, the parent taking care the child at preschool age is discordant with univariate results of association. (Table 7)

Table 6 The determinants of secondary dengue infection compared to naïve in school-aged children at Meuang district, Rayong province, Thailand

Factor	Unexposed case – Exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
Behavioural information				
Period 1: INFANTILE AGE (After birth to 1 year of age)				
• During infancy, a parent took care of the child.	3	5	0.60 (0.14, 2.51)	0.48
• During infancy, the child was staying at the current place.	11	7	1.57 (0.61, 4.05)	0.34
• During infancy, the child's habitat was located in an urban area (large city/city/town).	10	5	2.00 (0.68, 5.85)	0.19
• During infancy, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	6	4	1.50 (0.42, 5.32)	0.50
• During infancy, the child had a fever very often (> 10 times in a year).	0	0	undefined	
• During infancy, the child visited to hospital/clinic often (≥ 6 times/year) due to illness.	1	4	0.25 (0.03, 2.23)	0.18
• During infancy, the child had	2	3	0.67	0.65

Factor	Unexposed case – Exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
been diagnosed with any dengue disease by a medical doctor.			(0.18, 3.99)	
Period 2 : PRESCHOOL AGE (1- 5 years old)				
• During 1-5 years old, a parent took care of the child.	2	9	0.22 (0.04, 1.03)	0.03
• During 1-5 years old, the child was staying at the current place.	7	6	1.17 (0.39, 3.47)	0.78
• During 1-5 years old, the child's habitat was located in urban area. large city/city/town).	3	4	0.75 (0.16, 3.35)	0.70
• During 1-5 years old, the child never migrated from his/her home town.	4	4	1.00 (0.25, 4.00)	1.00
• During 1-5 years old, the child had a fever very often (≥ 3 times/year).	11	3	3.67 (1.02, 13.14)	0.03
• During 1-5 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	4	11	0.36 (0.11, 1.14)	0.07
• During 1-5 years old, the child had been diagnosed with any dengue disease by a medical doctor.	3	4	0.75 (0.16, 3.35)	0.70
• During 1-5 years old, the child attended nursery or kindergarten.	3	3	1.00 (0.20, 4.95)	1.00
• During 1-5 years old, the child attended the nursery or kindergarten in early age (≤ 3 years of age).	7	5	1.40 (0.44, 4.41)	0.56
• During 1-5 years old, the child never travelled to another province.	7	11	0.63 (0.24, 1.64)	0.34
• During 1-5 years old, in the daytime at home, the child often slept indoors.	9	10	0.90 (0.36, 2.21)	0.81
• During 1-5 years old, in the daytime at home, the child often slept under bed netting.	10	13	0.77 (0.33, 1.75)	0.53
• During 1-5 years old, in the daytime at home, the child often slept under an electric fan.	7	6	1.17 (0.39, 3.47)	0.78
• During 1-5 years old, in the daytime at home, the child often took a bath/shower before sleep.	9	10	0.90 (0.36, 2.21)	0.82
• During 1-5 years old, the child had ≥ 6 peers/closest friends in his/her community	7	2	3.50 (0.72, 16.85)	0.09

Factor	Unexposed case – Exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
• During 1-5 years old, the child spent > 6 hours per day indoors at home during the daytime.	10	7	1.43 (0.54, 3.75)	0.47
• During 1-5 years old, the child often had lunch at restaurant/outside the home.	7	12	0.58 (0.23, 1.48)	0.25
• During 1-5 years old, the child usually had dinner before 6 pm.	8	7	1.14 (0.41, 3.15)	0.79
Period 3: SCHOOL AGE 6-18 years old				
• During 6-18 years old, parent was taking care of the child.	3	7	0.43 (0.11, 1.65)	0.20
• During 6-18 years old, the child was staying at the current place.	2	2	1.00 (0.14, 7.10)	1.00
• During 6-18 years old, the child's habitat was located in an urban area (large city/city/town).	4	2	2.00 (0.36, 10.92)	0.41
• During 6-18 years old, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	6	0	undefined	
• During 6-18 years old, the child had fever very often (≥ 3 times/year).	10	8	1.25 (0.49, 3.17)	0.64
• During 6-18 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	5	2	2.50 (0.48, 12.88)	0.25
• During 6-18 years old, the child had been diagnosed with any dengue disease by a medical doctor.	4	6	0.67 (0.19, 2.36)	0.53
• During 6-18 years old, the child never travelled to another province.	10	8	1.25 (0.49, 3.17)	0.63
• During 6-18 years old, at weekend at home, the child often slept under an electric fan.	7	5	1.40 (0.44, 4.41)	0.56
• During 6-18 years old, at weekend at home, the child often slept under bed netting.	2	9	0.22 (0.05, 1.03)	0.03
• During 6-18 years old, at weekend at home, the child often took a bath/shower before sleep.	7	8	0.87 (0.32, 2.41)	0.79
• During 6-18 years old, the child had > 6 peers/closest friends in his/her community	3	2	1.50 (0.25, 8.98)	0.65
• During 6-18 years old, the child spent > 6 hours per day indoors at home during the daytime.	11	9	1.22 (0.50, 2.95)	0.65

Factor	Unexposed case – Exposed control (pair)	Exposed case - unexposed control (pair)	Match OR (95% CI)	P value
• During 6-18 years old, the child usually had dinner before 6 pm.	7	5	1.40 (0,44, 4.41)	0.56
• During 6-18 years old, the child never changed schools.	11	3	3.67 (1.02, 13.14)	0.03
• During 6-18 years old, the child took a tutorial class in the evening ≥ 3 times/week.	8	5	1.60 (0.52, 4.89)	0.40
• During 6-18 years old, the child had a classmate diagnosed with a dengue disease by a medical doctor.	2	1	2.00 (0.18, 22.05)	0.56
Current household information				
• The child lived with the care giver in the same place for more than 5 years.	9	5	1.80 (0.06, 5.37)	0.28
• The habitat was apartment/townhouse.	12	6	2.00 (0.75, 5.77)	0.15
• The common area in village such as temple, school, grocery store or convenience store was < 1 km distance from the child's habitat	4	4	1.00 (0.25, 4.00)	1.00
• Having domestic animal/pet	12	10	1.20 (0.52, 2.78)	0.67
• Having outdoor surrounding vegetation	3	4	0.75 (0.17, 3.35)	0.70
• Having indoor plants	8	6	1.33 (0.46, 3.84)	0.59
• Having tap water as a water supply	4	9	0.44 (0.14, 1.44)	0.17
• Having a shower in the child's bathroom	8	11	0.72 (0.29, 1.80)	0.49
• Having a water container in the child's bathroom	5	1	5.00 (0.58, 42.80)	0.10
• Having a toilet in the child's home	0	0	undefined	
• Child always uses bed netting.	6	8	0.75 (0.26, 2.16)	0.59
• Having unused tyres in the child's home area	5	4	1.25 (0.33, 4.65)	0.74
• Having discarded cans/ plastic/utensils in the child's home area	11	9	1.22 (0.50, 2.95)	0.65
• Having experience of mosquito bites during the daytime	0	2	undefined	
• Having mosquito larvae in the child's home	12	10	1.20 (0.52, 2.78)	0.67
Personal information				
• Male gender	7	11	0.64 (0.25, 1.64)	0.34

Table 7 Conditional logistic regression of risk factor for secondary dengue infection in school-aged children in the Meuang District of Rayong Province, Thailand

Factor	Adjusted OR	95% CI.		P-value
Parent took care the child during preschool age.	1.94	0.23	16.38	0.54
The child had fever very often (≥ 3 times/year) during preschool age.	5.94	1.00	35.27	0.05
The child never changed schools at school age.	5.00	0.86	28.93	0.07
The child often slept under bed netting at school age.	0.17	0.03	1.17	0.07

Risk and protective factors of all dengue infection compared to naïve participants

For all dengue infections (either primary or secondary), 7 significant determinants in all age periods including household factor were revealed. The risk factors included birth in an urban area (p=0.05), no migration from his/her home town during infancy (p=0.04), no migration from his/her home town during school age (p=0.02), no change in schools at school age (p=0.004) and having a water container in a bathroom (p = 0.003). The protective factors revealed were parental childcare during preschool age (p=0.03), the child often sleeping under bed netting during weekend at home in school age (p=0.04).

Only 3 risk factors were significant in conditional logistic regression analysis. These were birth in an urban area (p=0.02), no change in schools at school age (p=0.02) and having a water container in a bathroom (p = 0.02). In conditional logistic regression analysis, the protective factor of sleeping under bed netting at weekend during school age was a protective factor, but it was not statistically significant (p=0.09).

Table 8 The determinants of all dengue infection compare to naïve in school-aged children at Meuang district, Rayong province, Thailand

Factor	Match OR	(95% CI)		P value
Behavioural Information				
Period 1: INFANTILE AGE (After birth to 1 year of age)				
• During infancy, a parent took care of the child.	0.42	0.15	1.17	0.08
• During infancy, the child was staying at the current place.	1.18	0.56	2.50	0.66
• During infancy, the child's habitat was located in an urban area (large city/city/town).	2.33	0.91	5.96	0.05
• During infancy, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	3.00	1.06	8.48	0.04
• During infancy, the child had a fever very often (> 10 times in a year).	4.33	0.46	40.83	0.16
• During infancy, the child visited to hospital/clinic often (≥ 6 times/year) due to illness.	1.07	0.31	3.62	0.91
• During infancy, the child had been diagnosed with any dengue disease by a medical doctor.	0.70	0.14	3.62	0.67
Period 2 : PRESCHOOL AGE (1- 5 years old)				
• During 1-5 years old, a parent took care of the child.	0.26	0.07	0.96	0.03
• During 1-5 years old, the child was staying at the current place.	1.07	0.45	2.52	0.88
• During 1-5 years old, the child's habitat was located in urban area. large city/city/town).	0.89	0.28	2.79	0.84
• During 1-5 years old, the child never migrated from his/her home town.	1.22	0.47	3.22	0.68
• During 1-5 years old, the child had a fever very often (≥ 3 times/year).	0.53	0.22	1.25	0.13
• During 1-5 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	0.89	0.46	1.99	0.78
• During 1-5 years old, the child had been diagnosed with any dengue disease by a medical doctor.	0.59	0.16	2.22	0.42
• During 1-5 years old, the child attended nursery or kindergarten.	0.60	0.14	2.63	0.51
• During 1-5 years old, the child attended the nursery or kindergarten in early age (≤ 3 years of age).	1.76	0.80	3.85	0.15
• During 1-5 years old, the child never travelled to another province.	1.40	0.70	2.76	0.31

Factor	Match OR	(95% CI)		P value
• During 1-5 years old, in the daytime at home, the child often slept indoors.	0.85	0.43	1.68	0.64
• During 1-5 years old, in the daytime at home, the child often slept under bed netting.	0.70	0.35	1.41	0.31
• During 1-5 years old, in the daytime at home, the child often slept under an electric fan.	0.73	0.28	1.92	0.53
• During 1-5 years old, in the daytime at home, the child often took a bath/shower before sleep.	0.85	0.40	1.78	0.65
• During 1-5 years old, the child had ≥ 6 peers/closest friends in his/her community	1.87	0.65	5.40	0.23
• During 1-5 years old, the child spent > 6 hours per day indoors at home during the daytime.	2.04	0.90	4.58	0.07
• During 1-5 years old, the child often had lunch at restaurant/outside the home.	0.70	0.33	1.48	0.34
• During 1-5 years old, the child usually had dinner before 6 pm.	1.08	0.50	2.32	0.84
Period 3: SCHOOL AGE 6-18 years old				
• During 6-18 years old, parent was taking care of the child.	0.38	0.10	1.32	0.11
• During 6-18 years old, the child was staying at the current place.	1.14	0.20	6.55	0.88
• During 6-18 years old, the child's habitat was located in an urban area (large city/city/town).	1.50	0.37	6.00	0.52
• During 6-18 years old, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	3.89	1.15	13.08	0.02
• During 6-18 years old, the child had fever very often (≥ 3 times/year).	1.24	0.56	2.75	0.59
• During 6-18 years old, the child visited a hospital/clinic often (≥ 3 times/year) due to illness.	2.15	0.69	6.68	0.20
• During 6-18 years old, the child had been diagnosed with any dengue disease by a medical doctor.	0.60	0.17	2.02	0.41
• During 6-18 years old, the child never travelled to another province.	1.67	0.81	3.43	0.15
• During 6-18 years old, at weekend at home, the child often slept under an electric fan.	0.83	0.33	2.07	0.69
• During 6-18 years old, at weekend at home, the child often slept under bed netting.	0.35	0.12	0.99	0.04

Factor	Match OR	(95% CI)		P value
• During 6-18 years old, at weekend at home, the child often took a bath/shower before sleep.	0.86	0.40	1.84	0.68
• During 6-18 years old, the child had > 6 peers/closest friends in his/her community	1.53	0.45	5.23	0.48
• During 6-18 years old, the child spent > 6 hours per day indoors at home during the daytime.	1.28	0.60	2.72	0.51
• During 6-18 years old, the child usually had dinner before 6 pm.	1.60	0.60	4.26	0.34
• During 6-18 years old, the child never changed schools.	5.86	1.56	22.04	0.004
• During 6-18 years old, the child took a tutorial class in the evening ≥ 3 times/week.	1.88	0.74	4.66	0.16
• During 6-18 years old, the child had a classmate diagnosed with a dengue disease by a medical doctor.	1.50	0.17	13.42	0.70
Current household information				
• The child lived with the care giver in the same place for more than 5 years.	1.20	0.48	2.98	0.70
• The habitat was apartment/townhouse.	1.96	0.90	4.23	0.08
• The common area in village such as temple, school, grocery store or convenience store was < 1 km distance from the child's habitat	0.97	0.40	2.31	0.94
• Having domestic animal/pet	1.16	0.57	2.35	0.66
• Having outdoor surrounding vegetation	0.37	0.10	1.42	0.13
• Having indoor plants	1.71	0.67	4.38	0.26
• Having tap water as a water supply	0.54	0.22	1.31	0.15
• Having a shower in the child's bathroom	0.72	0.33	1.60	0.43
• Having a water container in the child's bathroom	13.00	1.58	106.73	0.003
• Having a toilet in the child's home	undefined			
• Child always uses bed netting.	1.56	0.67	3.62	0.32
• Having unused tyres in the child's home area	1.89	0.71	4.99	0.19
• Having discarded cans/ plastic/utensils in the child's home area	1.35	0.63	2.92	0.44
• Having experience of mosquito bites during the daytime	0.90	0.19	4.30	0.90
• Having mosquito larvae in the child's home	1.23	0.62	2.68	0.49
Personal information				
• Male gender	0.68	0.33	1.39	0.28

Table 9 Conditional logistic regression of risk factors for all dengue infections for school-aged children at the Meuang District of Rayong Province, Thailand

Factor	Adjusted OR	95% CI.		P-value
The infant's habitat location was urban.	8.57	1.41	52.01	0.02
The infant never migrated from his/her home town (defined as staying at least 1 month in a new place).	2.15	0.50	9.34	0.30
At preschool age, a parent took care of the child.	0.55	0.08	3.80	0.54
At school age, the child never migrated from his/her home town (defined as staying at least 1 month in a new place).	2.15	0.39	11.94	0.38
At school age, the child often slept under bed netting.	0.28	0.06	1.25	0.09
At school age, the child never changed schools.	18.64	1.66	209.08	0.02
Having a water container in a bathroom	94.60	2.17	4117.14	0.02

Discussion

The results demonstrate that the determinants of primary vs. secondary dengue infection are different when stratified according to the age period. The determinants of primary dengue infection were only found in the infantile period and the determinants of secondary dengue infection were only found in older age periods, especially in the preschool age and the school age periods. The findings strongly suggest that the first time of natural infection of dengue is in a younger age.

The results of primary dengue infection imply that they were infected for the first time while they were still infants. The strongest independent determinant of primary dengue infection in our study was the birth place located in an urban area. This factor was statistically significant in both bivariate and conditional logistic regression analyses. There are 2 possibilities that support this finding. Firstly, the chance of dengue infection in an urban area is higher than that of a rural in Thailand.[29] Secondly, the study area of the Meuang District of Rayong Province has had a high report rate over time (Figure 3) and has presented all 4 types of dengue. This is what is called "hyperendemicity with multiple dengue serotype circulation".[30] Moreover, the infants themselves were more prone to mosquito bite due to their lack of mobility and longer sleep times during the daytime compared with older age groups. Iglowstein et al. observed the sleep pattern among 493 Swiss babies and revealed that 1 month-old infants required 5-6 hours on average for daytime naps; 6 month-old infants required 3.4 hours; and 12 month-old infants needed 2.4 hours, but a 2 year-old child needed only 2 hours on average for the daytime sleep.[31] These finding are the firm supporting evidences that urban birth place location was the risk factor of primary dengue infection in the Meuang District of Rayong.

The second risk factor relevant for the infantile period was that of no migration from his/her home town (defined as staying for more than 1 month in a new place). Even though this factor was not significant (p=0.06) in conditional logistic regression analysis, its strength suggests it could play a role as a risk factor (adjusted OR = 8.02). However, the reason for people who stay in the same place having an apparently higher risk is unclear.

The result of primary dengue infection discovered in infantile age period is problematic in terms of control and prevention of dengue. This demonstrates a failure in control the disease. Once the child is born, they are infected in a very early age. Nonetheless, this finding may differ from other provinces since the Meuang District of Rayong Province has hyperendemicity and all 4 serotypes of dengue are in circulation. Delay in the first time of dengue infection has been observed in countries that have very good control and prevention measures such as Singapore. This country has implemented a complete vector control programme since 1973. Three main intervention and control measures have included source reduction since 1968[32], public education and law enforcement by the Destruction of Disease Bearing Insects Act of 1968[33]. The law enforcement has been the strongest of any South East Asian country. The program has been focusing on households and institutions, successfully reducing the mosquito larval index in human habitats to approximately 2% by 1973.[33] The successful programme has led to Singaporean children, having lowered dengue immunity and delayed first time dengue infection in childhood. We can observed that dengue cases in Singapore were mainly dengue fever and affected adult.[ref] For example, in 2003, Singapore reported a proportion of dengue fever of 30 % in adults (age ≥ 25 years)and this proportion increased to 70% by 1999.[34] The increasing trend of age among dengue cases suggests that vector control has been improving over the years. This phenomenon has also been observed in Thailand (Figure 6). However, in contrast to Singapore, the change in this finding in Thailand has been gradual. In conclusion, the results of our study reveal that the children in the Meuang District of Rayong Province may be infected while they are infants. Once we have the effective vector control implementation, we can delay the first time of infection at infantile age. To achieve this goal, a re-evaluation of the vector control programme must be undertaken in this area.

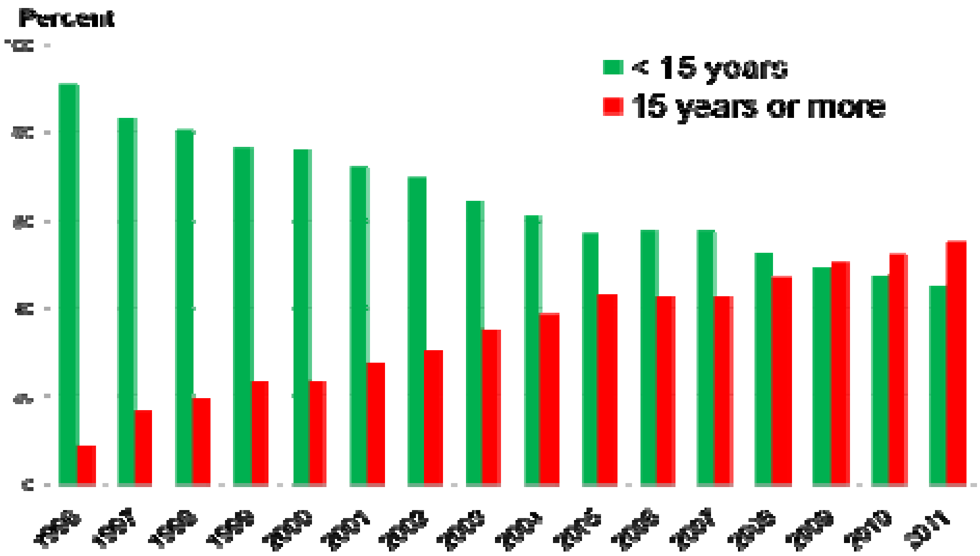

Figure 8 Proportion of all dengue cases (DF/DHF/DSS) between children (below 15 years of age) and adults (over 15 years of age) from 1996 to 2011, Thailand.

Source: The Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health.

The determinants of secondary dengue infection were only found at the preschool and school age periods. There was no association between secondary dengue infection and behavioural determinants at infantile period in both univariate and multivariate analyses. The predictor of secondary dengue infection is the child having fever very often (≥ 3 times per year on average) at preschool age. This factor was found to be significant in univariate analysis and was the strongest in multivariate analysis. This result may have been because secondary dengue infection was more likely to present with this symptom.[35] On the other hand, this factor was not statistically significant in the primary dengue infection analysis. It may be because primary dengue infection has less clinical presentation of this symptom than secondary infection. This finding will be useful for the paediatrician to ask about the frequency of fever at preschool to predict secondary infection. There is a need to predict severe dengue infection in very early of clinical presentation and on admission because secondary dengue infection tends to have a serious outcome. A history of high frequency of fever episodes may not be entirely sufficient to predict severe dengue infection. Thus, the paediatrician must evaluate the other warning signs of severe dengue in the revised WHO case definitions, 2009.[36] This factor should be further researched in a different area to confirm its association with secondary dengue infection.

The other risk factor for secondary dengue infection was the child never having changed schools at school age. This factor was found to be significant in univariate (p=0.03) analysis, but it was not significant in multivariate analysis by conditional logistic regression (p=0.07). The explanation for why the child who studied in the same school was at higher risk than other children who changed schools remains unclear.

The protective factor for secondary dengue infection was sleeping under bed netting during the daytime. Even though this factor was not significant in multiple logistic regression analysis (p=0.07), this factor is considered in theory to protect against dengue. Thus, this factor will play a role as a preventive measure for secondary dengue infection. In recent times, many methods to prevent mosquito bite during the daytime have become available. These are chemical sprays, chemical repellents such as DEET and picaridin, chemical mosquito coils, and many type of Ovitraps. Nevertheless, bed netting is still an effective measure to prevent mosquito bites while the child is asleep. Its benefits include low cost and its availability to all households. Therefore, bed netting is still effective in preventing mosquito bites in this area.

In our study, we also evaluated the prevention capacity of an electric fan, but it was not significant in any type of dengue infection. Nonetheless, its result was somewhat in the protective direction (OR < 1) in all dengue infections and in all age periods. In theory, an electric fan can prevent mosquito bites by 2 methods: 1) winds disperse the carbon dioxide which is a chemotaxis of mosquito; and 2) winds interfere with the flight of the mosquito.[37]

A parent as a caregiver in preschool age revealed significance as a protective factor from secondary dengue infection by univariate analysis. However, by conditional logistic regression analysis, this factor was not associated with secondary dengue infection (p=0.54).

The determinants of all dengue infections; namely, primary and secondary dengue infections, were positive in all age groups(infantile, preschool and school age), including household determinant. Seven determinants were statistically significant. These findings were quite similar to the primary and secondary infection results. These were urban location of the infant's birth place (also in primary infection), no migration from his/her home town (also in primary infection), parental childcare during preschool age (also in secondary infection), no migration from his/her home town during school age, the child often sleeping under bed netting during the weekend at home in school age (also in secondary infection), the child never having changed schools during school age (also in secondary infection) and having a water container in the bathroom. Only 3 risk factors revealed significance in conditional logistic regression analysis. These were urban location of the infant's birth place (p=0.02), the child never having changed schools at school age (p=0.02) and having a water container in a bathroom ($p = 0.02$). The interesting determinant in all dengue infections was a household characteristic because the household information was not associated in both analyses of primary and secondary infection. This may have been because of the low sample size in the analysis. Having a water container in the bathroom is common in Thailand. Water containers in bathrooms are used for taking a bath or latrine cleaning. A water container in a bathroom is a habitat for mosquito infestation. Many studies in Thailand demonstrate that the bathroom container is a key container of *Aedes* mosquito.[38,39] Thus, an intervention must carefully evaluate the mosquito larval index of the water reservoir in the bathroom. This information must be shared with the village health volunteer with the aim of increasing the awareness of the inspector.

Having fever very often in preschool age was significant in secondary dengue infection, but it was not significant in all dengue infections. It may have been diluted by primary dengue infections. This factor may only play a role in secondary infection.

The reason for the higher risk of all dengue infections associated with the child never having changed schools compared with the child who had changed schools is still unclear. If this is a true risk, it may imply that the child is first infected at home during infantile age (primary dengue infection), and then the second infection may occur in the school where the child studies longer than other children.

Not all significant determinants found in both primary and secondary dengue infections will be positive in all dengue infections. For example, having fever very often during preschool age was the risk factor for secondary dengue infection, but it was not the risk factor in all dengue infections. On the other hand, some determinants were found in all dengue infections, but when stratified into primary and secondary dengue infection, they were not determinants. For example, having a water container in the bathroom was a risk factor of all dengue infections, but it was not positive for both primary and secondary dengue infection. This may have been because of the limitation in our sample size .

Limitations in this study include recall bias. This bias is a crucial potential limitation in our study because the guardians or caregivers of the students might have forgotten the habits of the adolescent participants, especially at the infantile and preschool ages. Furthermore, some caregivers or guardians of an individual child were a different person among the 3 age groups. This may have resulted in inaccuracy of the information. Moreover, we were not able to interview all caregivers or guardians of the students. On the other hand, the activities of the children in adolescent age could be asked directly to the students. Therefore, they could give more precise information than the guardians or parent. We suggest that a further study should interview both caregivers or guardians and students. Our study revealed that the first time of infection (primary infection) may have occurred at infantile age, but the result should be treated with caution in interpretation because our study was a retrospective study with recall bias. We suggest that a further prospective cohort study of risk factors for dengue infection after birth to 1 year old be conducted in this area to confirm our finding of first infection at infantile age. We matched the school location between cases and controls. Thus, the socioeconomic status of participants may have been indirectly matched and created similarity among cases and controls. The sample size of our study is relatively low, but it is sufficient to test the hypotheses. The problem was that we were not able to find a valid control who was naïve of dengue infection to match with the randomized case. A long list of questions could make the interviewers and interviewees exhausted and resulted in invalid answers for some questions.

Conclusions and recommendations

Our study confirmed that there are different determinants playing a role at primary and secondary dengue infection. Primary dengue infection was associated with determinants occurring at infantile age and secondary dengue infection was associated with determinants occurring at older ages. Determinants of all dengue infections, including household characteristics, were revealed in all age groups.

In conclusion, 5 independent risk factors were identified in our study. One risk factor was found in each of primary and secondary dengue infection. Three risk factors were found in all dengue infections. The only one important risk factor of primary dengue infection occurred at infantile age. This was urban location of birth place in the Meuang District of Rayong Province. The only one strong risk factor for secondary dengue infection was found in preschool age. This was having fever very often ($\geq$ 3 times per year) at preschool age. The three important risk factors in all dengue infections were found to include urban birth place in the Meuang District of Rayong Province, the child never having changed schools at school age and having a water container in a bathroom.

From our study finding we can conclude that the prevention of dengue infections must target children in all age groups. The campaign should not focus only on school age children and neglect infants. Two main interventions must be done in this study area. These are health education to be given to the public to prevent mosquito bites, and promotion of measures to reduce mosquito larvae sources. Law enforcement is less likely to be practical in Thailand.

The recommendations for prevention of primary and secondary dengue infections should be emphasized in all age groups.

Prevention for Dengue Infections

1. The result of this study found that the child who was born in an urban area and had stayed at that place for over a year was at risk of first time infection. Therefore, prevention during infantile age should include health educational messages for pregnant women to prevent mosquito bites in their future infants. Additional health information about how to prevent mosquito bites for her baby must be emphasized at the antenatal care unit (ANC). This additional work can be done by a nurse at the ANC unit in health centres. All pregnant women can receive health education for dengue preventions up to 4 times while attending scheduled ANC before delivery. Brochures and pamphlets showing how to prevent mosquito bites in babies can be given to raise awareness among pregnant women. These educational materials consist of DO and DON'T points for the pregnant woman. The national programme of ANC must integrate dengue prevention into the existing activities. Messages for prevention of mosquito bites must be shown on a poster and put on the wall in the area visible to pregnant women in the ANC unit.

2. The health education will be addressed again while the pregnant woman is admitted to hospital for her delivery. After delivery, an infant will be admitted with her/his mother for at least 3 days in uncomplicated cases. This is the golden period for demonstrative health education by health promotion staff working at the health promotion unit. They must provide the important message of how to prevent mosquito bites during care of the baby at hospital and at home. Before discharging the child from the new-born ward, staff must ask the health promotion staff to visit mothers and give health education.

3. The health education can be provided at immunization clinic. Thai infants are scheduled for vaccination at 2, 4 and 6 months of age. Thus, in these visits the health providers who work at immunization clinic in health centres and/or hospitals must give health education to caregivers of children. For convenience, education messages on prevention of mosquito bites are given to groups of caregivers while they are waiting for child vaccination as well as additional messages on posters on the walls of the clinic.

4. Cleaning water containers and destroying mosquito larvae in the homes of pregnant women and mothers is essential. This mandatory measure must be inspected and encouraged by a village health volunteer and health staff during home visits.

5. The secondary dengue prevention will focus on preschool and school age children. The health education of dengue and mosquito must be integrated with other disease prevention at day-care centres and kindergarten schools. In Thailand, hand foot and mouth disease (HFMD)

prevention is widely known among teachers; thus, the message of prevention of mosquito bites must be integrated with HFMD prevention education. This is highly feasible because the season of HFMD and dengue diseases in Thailand is in the rainy season during the months of May to September.

6. For prevention of dengue infections (primary and secondary), special attention must be paid to cleaning and disposing of the containers which are the habitat of mosquito larvae. Household containers must be inspected by village health volunteers, especially in and nearby households with children.

7. Education about dengue prevention must be taught at school in all grade levels. Knowledge of dengue risk reduction must be compulsory for Thai students. To improve attitudes among students, good practice of mosquito prevention in students' homes should be linked to extra behaviour marks for students' school grades. Normally, the students in Thailand have 10 marks out of 100 for their school grades. This technique may improve the students' attitudes and practices.

8. Conducting an evaluation of dengue disease surveillance in the Meuang District is useful. We suspect that the first time of dengue infection occurred at infantile age, and this likely reflects a weakness of the surveillance and response system. Health officers must look for dengue cases in the young aged population.

9. Adding a criterion for outbreak investigation in dengue diseases should be considered by the Bureau of Epidemiology of the Department of Disease Control. Once an infant case of dengue infection is identified, health officers must start a case investigation. Currently, only three criteria are required for dengue investigation including 1) a fatal dengue case, 2) a cluster or outbreak of dengue diseases and 3) first case(s) of dengue reported in a community. Therefore, we recommend adding a dengue case in an infant as a new criterion for investigation.

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