PREVALENCE AND CHARACTERISTICS OF DENGUE AND CHIKUNGUNYA INFECTIONS AMONG ACUTE FEBRILE PATIENTS IN NONG KHAI PROVINCE, THAILAND

Sujet Lertanekawattana[1], Surapee Anantapreecha[2], Chuleeporn Jiraphongsa[3], Pawinee Duan-ngern[3], Sathit Potjalongsin[1], Wisanu Wiittayabamrung[4], Pamol Daroon[4] and Meta Techolarn[4]

[1]Nong Khai Hospital, Ministry of Public Health, Nong Khai; [2]Department of Medical Science, Ministry of Public Health, Nonthaburi; [3]Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Nonthaburi; [4]Nong Khai Provincial Health Office, Ministry of Public Health, Nong Khai, Thailand

Abstract. We conducted a cross sectional study at three hospitals of Nong Khai Province, Thailand to determine the prevalence and characteristics of dengue and chikungunya infection among patients who sought care. The study population was acute febrile patients who visited these hospitals during 1 August -31 October, 2010 who were aged 2-60 years and had clinical symptoms compatible with the case definition. Dengue and chikungunya cases were confirmed by an ELISA IgM titer or RT-PCR. We also reviewed surveillance data of dengue and chikungunya infections from 2003-2009. Of the 200 participants recruited into the study, 103 patients (51.5%) were confirmed to have acute dengue infection; dengue serotype 2 was the most prevalence serotype. The ages of confirmed dengue cases ranged from 2-37 years old. The distribution of cases showed that dengue morbidity tended to be clustered in adjacent areas, particularly in Mueang District. Only a small proportion of the patients uses mosquito repellant and had screens on their windows. One patient (0.5%) had laboratory confirmed chikungunya infection. She was from Rattanawapi District, an area where no chikungunya had been reported before. Since the disease varies by age and geographic location, increased awareness of health care workers and public health officers about the diseases in the area is needed for early detection of cases and to promote early prevention and control measures.

Keywords: dengue, chikungunya, surveillance, dengue serotype, Thailand

INTRODUCTION

Dengue and chikungunya infections are vector borne diseases causing substantial public health problems in Thailand. The diseases are transmitted by Aedes mosquitoes (Hochedez *et al*, 2006). Clinical manifestations during the early phase

Correspondence: Chuleeporn Jiraphongsa, Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Nonthaburi 11000, Thailand.
Tel: +66 (0) 2591 8581
E-mail: chulee@health.moph.go.th

are similar: fever, headache and joint pain. Both diseases are difficult to differentiate from other acute febrile illnesses prevalent in Thailand, such as leptospirosis, scrub typhus, and influenza (WHO SEARO, 1999). A study conducted in Thailand in 2001 revealed 10.7% of patients aged >15 years who presented with fever were confirmed to have dengue infection (Suttinont *et al*, 2006). Population movements are one factor influencing transmission of diseases across borders. For example, chikungunya crossed into Thailand in 2008-2009 (Ditsuwan *et al*, 2011) and spread from southern Thailand to other parts of the country. Other outbreaks of dengue and chikungunya infection have been spread by travelers to endemic areas (Hochedez *et al*, 2006; Simon *et al*, 2007; Heddini *et al*, 2009; Pistone *et al*, 2009).

Dengue surveillance has been conducted in Thailand for more than 40 years. It is a passive surveillance system. Cases are reported mostly based on the doctor's diagnosis. This study was conducted in Nong Khai Province, northeastern Thailand, and shares a border with Lao PDR. Dengue is a priority disease in Nong Khai Province; case investigations and control measures must be conducted within 48 hours. During 2003-2009, dengue morbidity in Nong Khai Province was 24.48 cases per 100,000 persons per year (range 13.78-35.71). In 2009 there were 376 reported cases of dengue infection in Nong Khai Province (Bureau of Epidemiology, 2010) which is twice the number of cases reported in 2008 (178 cases) (Bureau of Epidemiology, 2009).

Chikungunya surveillance was officially started in 2008 following a country wide epidemic of 2,494 cases, and by 2009 this number had drastically increased to 52,057 cases (Suangtho and Ouppapong, 2010). Reporting of chikungunya is also passive. Nong Khai Province reported two confirmed and 88 suspected chikungunya cases in 2009; the outbreak occurred in Phon Charoen District, where the index case was a resident who travelled to an epidemic province (Office of Diseases Prevention Control 6, 2009). This study was a part of a research project to assess the prevalence and epidemiology of dengue and chikungunya infection among patients with acute febrile illness who sought care at hospitals along the Thai-Lao PDR border.

MATERIALS AND METHODS

Design and study population

We conducted a cross sectional study at three hospitals in Nong Khai Province, consisting of a provincial hospital (Nong Khai) and two community hospitals (Phon Phisai and Phon Charoen). Nongkhai Hospital is a tertiary care hospital with 349 beds. Phon Phisai, a 60 bed hospital and Phon Charoen is a 30 bed hospital.

The study population was patients who presented to the study hospitals during 1 August - 31 October, 2010, who were aged 2-60 years and had clinical symptoms compatible with a case definition. The case definition was adapted from the WHO dengue case definition (WHO, 1999) but modified to increase the sensitivity and decrease the specificity to cover chikungunya infection. A suspected dengue case was defined as a patient who had fever (body temperature >37.8^{o}C) and had at least one of the following symptoms: ocular pain, malaise, arthralgia, rash, signs of dengue hemorrhagic fever (DHF) or dengue shock syndrom (DSS). DHF was defined as a suspected dengue case who had at least one of the following criteria: 1) a positive tournequet test, 2) thrombocytopenia (a platelet count <100,000 cells/m^3),

3) evidence of petechiae, ecchymosis or purpura, 4) evidence of bleeding (epistaxis, hematemesis), or 5) evidence of plasma leakage (a 20% increase in the hematocrit from baseline or sign of plasma leakage, such as ascites, plural or effusion) (WHO, 1999). Confirmed dengue or chikungunya infection was a suspected case who had an ELISA IgM titer > 1:40 or RT-PCR positive.

We excluded patients who had clinical symptoms or laboratory tests proving other infections such as acute otitis media (AOM), acute exudative tonsillitis, acute pharyngotonsilitis, urine analysis (UA) showing >10 WBC/HPF, chest x-ray showing an infiltration in the lung as well as a chief complaint consistent with pneumonia (fever, cough and dyspnea) or the patient was not willing to participate in the study.

We also reviewed surveillance data for dengue during 2003-2009 and all chikungunya surveillance data available from the provincial health office.

Diagnostic laboratory methods

Blood samples were obtained from the eligible study population. Dengue and chikungunya infections were confirmed by RT-PCR and IgM capture ELISA. Blood specimens were collected in tubes with EDTA; plasma was separated by centrifuge and kept at -20ºC. Specimens were transferred in dry ice to the Arbovirus Laboratory, Thai National Institute of Health (NIH) for laboratory testing. Blood sample of patients who presented with a fever for less than or equal to 5 days from onset of illness were tested with RT-PCR. For patients who presented after 5 days an appointment was made 14 days after the onset of fever and tested for IgM with an ELISA.

ELISAs for IgM and IgG antibodies were performed as previously described (Innis *et al*, 1989). In brief, 96-well U-bottom microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with anti-human IgM or anti-human IgG (Cappel, Aurora, OH). Fifty microliters of 1:100 diluted plasma samples or control samples were added in duplicate, washed 6 times with PBS-T, and 50 l of dengue viral antigen was then added. Twenty-five microliters of diluted horseradish peroxidase (HRP) human anti-flavivirus IgG conjugate (Thai NIH) were added. After washing 6 times with PBS-T, 0.5 mg/ml of OPD (O-phenylenediamine dihydrochloride, Sigma, St Louis, MO) with 3.3 l/ml of 3% H_2O_2 was added. Color development was stopped by addition of H_2SO_4, and the OD was read at 490 nm using a microplate autoreader (Bio-Tex instrument model EL 311, Bio-Tex instruments, Winooski, VT). The antibody levels were calculated from OD values as reported previously (Anantapreecha *et al*, 2007).

RT-PCR for dengue was performed as previously reported with some modifications (Yenchitsomanus *et al*, 1996) and RT-PCR for chikungunya was performed following the methods of Parida *et al* (2007). Briefly, RNA was extracted from 100 l of plasma using a QIAamp viral RNA mini kit. RT and PCR were performed in one tube using universal primer and a one-step RT kit (QIAGEN, Hilden, Germany). The reaction tube was placed in a thermal cycler machine (Perkin-Elmer-Cetus, Norwalk, CT). The primary PCR product was further used for nested PCR in another reaction tube. The nested PCR reaction tube was set in a thermal cycler. The secondary PCR product was subjected to agarose gel electrophoresis. Amplified DNA fragments were visualized after ethidium bromide staining. Serotypes were tested by serotype-specific RT-PCR (Sa-ngasang *et al*, 2003).

Sample size calculation

We calculated the sample size using the following formula:

$N = Z^2 (pq)/d^2$

where Z = 1.96, p = prevalence of the disease in the population, q = 1-p, d = margin of error (0.04). Dengue infection prevalence from previous study was 0.11 (Suttinont *et al,* 2006), and chikungunya infection was 0.13 (Office of Diseases Prevention Control 6, 2009). Therefore, the total sample size needed was approximately 200. We divided samples by the three hospitals based on the number of the patient visits per day. Nong Khai Hospital was given 100 cases (50 pediatric and 50 adult cases), Phon Phisai Hospital was given 50 cases, and Phon Charoen Hospital was given 50 cases. During the study period each site recruited eligible participants until the sample size was achieved.

Data collection and analysis

Doctors screened the patients who met case definition in either the outpatient department (OPD) or inpatient department (IPD). When a patient was willing to participate in the study, a face to face interview was conducted by a nurse using a structured questionnaire. Demographic information (age, gender, occupation, underlying disease), clinical symptoms, complete blood count (CBC) result and risk factors (history of family member having had dengue/chikungunya infection, the use of mosquito protection) was collected. Categorical data were presented as numbers and percentages, while continuous data were presented as means and standard deviations, medians, minimums, maximums and inter-quartile ranges (IQR). The chi-square test was used to compare categorical data, and the *t*-test or Kruskal-Wallis test were used to compare means and medians.

Ethical considerations

The study was approved by the ethics committee of Nong Khai Hospital. Participants or parents of participants aged <15 years are written consent prior to participation.

RESULTS

During the study period, 200 participants were included in the study, of which 99, 51, and 50 participants came from Nong Khai Provincial Hospital, Phon Charoen Hospital, Phon Phisai Hospital, respectively. Of the 200 cases, 99 cases (49.5%) were from the IPD and 101cases (50.5%) were from the OPD. The median length of time from symptom onset to hospital visit was 3 days (range 0-5, IQR 3-4 days). One hundred three patients (51.5%) had laboratory confirmed acute dengue infection and one patient (0.5%) had laboratory confirmed chikungunya infection. There was one death, from dengue giving a case fatality rate of 1%.

Dengue infection

Diagnostic of dengue infection could vary by case definition. Using our study definition, 103 cases (51.5%) were confirmed as having dengue infection. However, when we applied the WHO definition, 155 cases were classified as having suspected dengue infection and among these 83 cases (53.5%) had laboratory confirmed dengue infection. Using our study criteria 51 patients (25.5%) had DHF, but using the WHO case definition, 49 of 155 suspected dengue cases (31.6%) had DHF. The WHO dengue case definition (WHO,1999) was able to identify 83 cases from 103 positive laboratory for dengue infection or overall sensitivity of 80.6% (Table 1).

Table 1
Number of suspected dengue cases by definition and laboratory results, Nong Khai Province, 1 August-31 October 2010 (N = 200).

Category	Study definition		WHO definition[a]	
	Suspected cases	Positive laboratory (%)	Suspected cases	Positive laboratory (%)
DF	149	70 (47.0)	106	54 (50.9)
DHF	51	33 (64.7)	49	29 (59.2)
Others	0	0	45	20 (44.4)
Total	200	103	200	103

[a]WHO, 1999.

Table 2
Characteristics of suspected and confirmed dengue cases, Nongkhai Province, Thailand, 1 August - 31 October 2010.

Characteristics	Confirmed dengue (N=103)	Suspected dengue (N=97)	p-value
Hospitalization	74 (71.8%)	25 (25.8%)	<0.01
Median age (IQR)	13 (2-37)	10 (2-60)	0.03
Median hematocrit at first visit (IQR)	40.1 (37.0-42.8)	38.6 (35.6-40.1)	<0.01
Median platelet count at first visit (IQR)	117,000 (75,750-170,250)	218,500 (140,750-256,750)	<0.01
Median WBC count at first visit (IQR)	3,555 (2,908-4,712)	5,300 (3,500-7,575)	<0.01
WBC count <5,000 cells/mm^3	82 (80.4%)	45 (46.9%)	<0.01
Percent of lymphocytes >40%	79 (77.5%)	64 (66.7%)	0.09

Of the 103 confirmed dengue cases, the proportions of females and males were similar (50 females and 53 males). Median age of the cases was 13 years old (range: 2-37; IQR: 9-16). The majority of cases were school age children (age group 10-14 years: 41.8%; ages 15-19: 22.3%; ages 5-9: 20.4%). Most of the cases (77.7%) were students followed by preschool children (9.7%), employees (8.7%), farmers (1.9%) and others (1.9%).

The clinical symptoms and signs in the cases included fever (100%), headache (81.6%), myalgia (64.1%), arthralgia (28.2%), ocular pain (24.3%), rash (17.5%), epistaxis (17.5%), gastrointestinal (GI) bleeding (17.5%), petechiae (8.7%), bleeding gums (1.9%) and upper respiratory tract infection (1.9%). One case developed DSS and died. The majority of cases (71.8%) were hospitalized. Nearly all the cases (97.1%) improved except for one death (case fatality rate 1%) and 2 that could not be followed up (1.9%). The median length of hospital stay was 2 days (range: 1 -21; IQR: 1-5 days). Confirmed cases had higher hematocrits and lymphocyte counts, and lower platelet counts at the first visit compared to laboratory negative cases (Table 2).

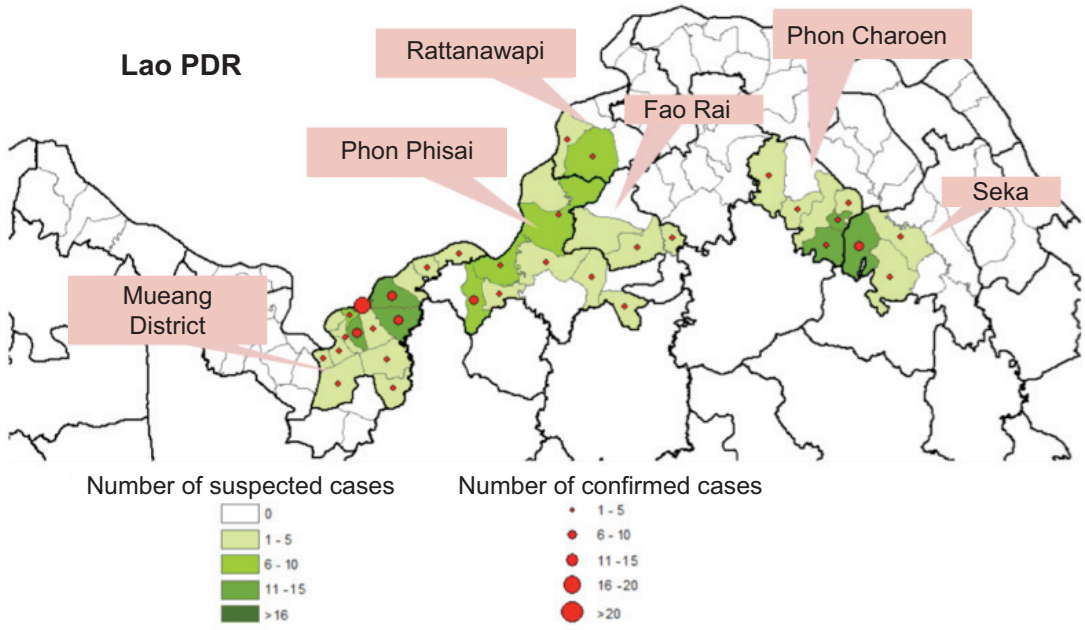

Fig 1–Number of suspected and confirmed dengue cases by subdistrict, Nong Khai Province, Thailand, 1 August-31 October 2010.

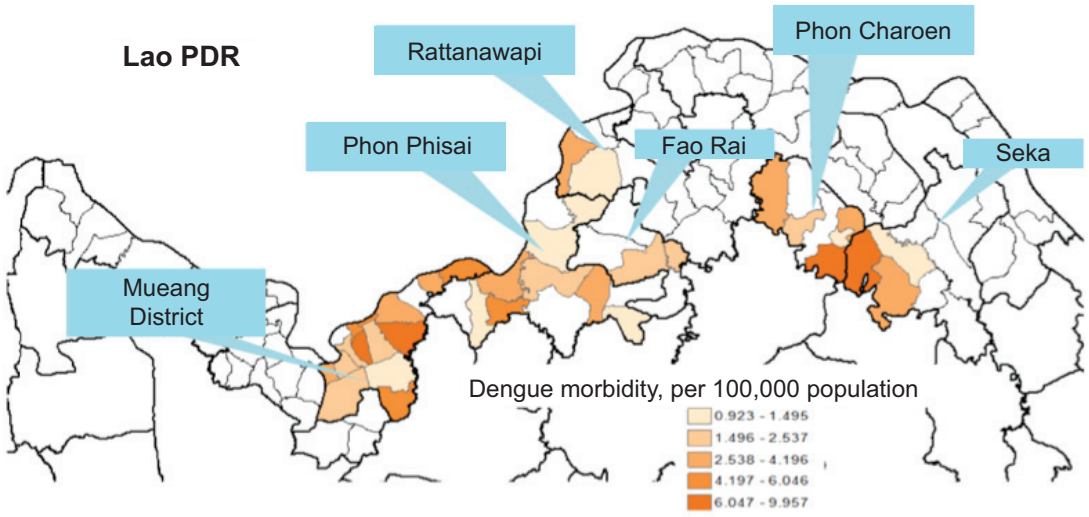

Fig 2–Dengue morbidity (per 100,000 population) by subdistrict, Nong Khai Province, Thailand, 1 August-31 October 2010.

Dengue confirmed cases had a slightly higher proportion of family members, neighbors, or classmates with dengue infection in the 2 week period prior to their illness than suspected cases but this was not significant (53% *vs* 42%, *p*-value =0.15). Confirmed cases were slightly more likely to have traveled to garden areas than suspected cases, but this was not significant (23% *vs* 19%). Confirmed and suspected cases had similar percentages who used mosquito repellant (8% *vs* 9%), used screens on their windows (26% *vs* 28%) and bed nets (83% *vs* 80%).

The percent of confirmed cases among all suspected cases at each hospital were not significantly different from each other (57.6%, 41.2%, and 50% at Nong Khai, Phon Charoen, and Phon Phisai Hospitals, respectively) (p=0.16). The distribution of cases by subdistrict showed the cases came from nearly every subdistrict in the study areas (Fig 1). Subdistricts with a large dengue morbidity tended to be clustered in some areas: Mueang District, and Phon Charoen District and Seka District (Fig 2).

The surveillance data showed a decreasing trend in dengue cases after the last week in July; however, there was a peak in cases in mid-August and cases continued until the end of September (Fig 3).

Chikungunya infection

Only one confirmed chikungunya case was identified in Ratanawapi District, where no chikungunya had been reported before. An investigation was conducted two days after receiving the laboratory results. The patient was a 13 year old girl who developed high fever, myalgia, and arthralgia on 4 August 2010. She visited hospital on 6 and 8 August. A complete blood count (CBC) was normal. She gave a history of visiting her cousin who lived nearby and had just come back from Lamae District, Chumphon Province where an outbreak of chikungunya was reported. Her cousin had fever with arthralgia on 25 July 2010. Her friend, who lived in the same house as her cousin, developed fever on 30 July 2010 and was diagnosed as having DHF at Nong Khai Hospital (not included in this study). The case had no history of travel to other areas. No additional cases of chikungunya were found in the village through active surveillance.

Laboratory exams

Two hundred samples were sent for investigation to the Thai NIH: 187 samples were tested with RT-PCR and 14 were tested with ELISA. One case was tested with both RT-PCR and ELISA. Of the 187 samples tested with RT-PCR, 98 samples (52.4%) were positive for dengue infection and 1 was positive for chikungunya infection. Of the 14 samples tested with ELISA, 5 (35.7%) were positive for acute dengue infection and nine were negative. None of the 14 samples was positive for chikungunya infection. Of the 5 positive ELISA cases, 4 were positive for IgM and IgG indicating either a secondary infection or a late blood collection, and 1 case was positive for IgM but negative for IgG indicating primary infection. Two of the nine specimens negative for IgM were positive for IgG, indicating a previous dengue infection.

In our study the age group with the highest proportion of positive tests for dengue was the 15-19 year old group (74.2%), followed by 10-14 years (63.2%), 20-24 years (57.1%), 20-34 years (50.0%) and 35-39 years (42.9%) (Fig 4). The RT-PCR was more likely to be positive if collected earlier in the infection, but

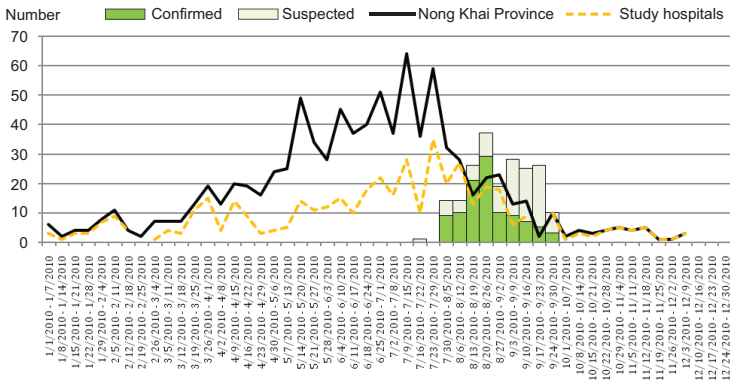

Fig 3–Distribution of dengue cases by date of onset from surveillance data (line) and study results (bar), Nong Khai Province, Thailand, 1 August-31 October 2010.

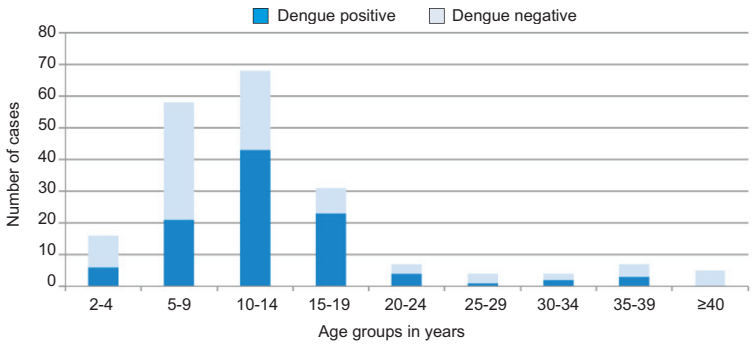

Fig 4–Dengue laboratory results by age group, Nong Khai Province, Thailand, 1 August-31 October 2010 (*N*=200).

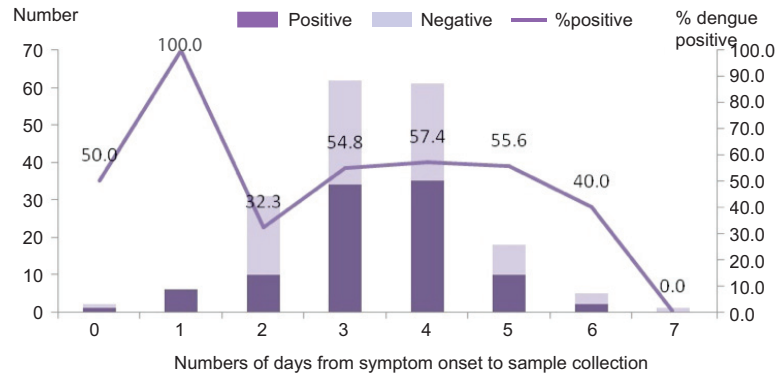

Fig 5–PCR for dengue infection results by day since onset of symptoms.

declined to approximately 50% after the third day of fever; was less than 40% on day 6 and 0% on day 7 (Fig 5). Dengue serotype 2 was the most prevalent serotype (68.4%) followed by serotype 1 (29.6%), serotype 3 (1%) and 4 (1%). Dengue serotype 3 was identified in the patient who died.

DISCUSSION

Fifty-one point five percent of patients presenting with acute febrile illness and having at least one symptom from the WHO's case definition for suspected dengue infection (WHO, 1999) were laboratory confirmed to have dengue infection. Our results are higher than a previous study conducted in Thailand (Suttinont *et al*, 2006) and Bangladesh (Faruque *et al*, 2012). The case fatality rate in our study of 1% was also higher than the case fatality rate reported from national disease surveillance (Sanohseang and Ouppapong, 2010).

Similar to the data from the dengue national disease surveillance and a study conducted in Bangkok and Ratchaburi Provinces (Tuntaprasart *et al*, 2003; Bongsebandhu-phubhakdi *et al*, 2008; Sanohseang and Ouppapong, 2010), school age children and teenagers

(aged 10-14, 5-9, and 15-19 years) were the most affected population. Dengue morbidity was high and clustered in the urban subdistricts of each district, similar to another study conducted in Thailand and in Vientiane, capital city of Lao PDR (Strickman *et al*, 2000; Vallee *et al*, 2009).

The data from the passive laboratory surveillance of the National Institute of Health shows a variety of dengue serotypes in different regions of Thailand. Dengue serotype 1 was found to be the most common serotype for all four regions of Thailand in 2009 (Sanohseang and Ouppapong, 2010). Our study found dengue virus serotype 2 was the most common serotypes (68.4%). The relationship between dengue serotype and severity of disease is not clearly understood. A study in Bangkok, Thailand and Indonesia found dengue serotype 3 was most often associated with severe and fatal infections (Nisalak *et al*, 2003). In our study serotype 3 was confirmed in the case who died. However, our study had a small sample size; hence, further studies need to determine the association between dengue serotype and severity of disease.

Compatible with the WHO guidelines for dengue diagnosis (WHO, 1999), our study found confirmed dengue cases had a higher hemotocrit, a lower platelet count and lower white blood cell count than laboratory negative cases. We also found confirmed cases were slightly more likely to report having family members or neighbors with dengue within two weeks of developing their own symptoms. Only a small proportion of patients used mosquito repellant or had screens on their windows.

The clinical manifestations of dengue fever and chikungunya fever are similar, which makes it difficult to differentiate between the two diseases clinically. A study in Thailand found rash, myalgia/arthralgia, and conjunctival injection were presented more frequently in chikungunya patients (WHO SEARO, 1999). Our study found only one case of chikungunya infection (0.5%) among acute febrile patients, which is lower than a study conducted in Nong Khai in 2009 after an epidemic of chikungunya in Phon Charoen District in June 2009 (Office of Diseases Prevention Control 6, 2009). The case investigation suggested the patient might have become infected from her cousin who had traveled to an epidemic area. However, the source of infection was inconclusive because no further laboratory investigations were conducted among the patient's close contacts.

Our findings show the RT-PCR test was more likely to be positive result when the specimens were collected during the early days of fever (days 1-4) and less likely to be positive after day 5. These results are comparable to another study from Thailand (Sa-ngasang *et al*, 2003).

Our study had some limitations, such as selection bias which could have occurred during case screening, since the doctor might have wanted to include the patient in the study, therefore, the proportion of patients with dengue infection among the acute febrile patients might be overestimated. Our sample size might be too small to detect the low prevalence of chikungunya. Our study was conducted during the late rainy season; therefore, we could not fully determine the epidemiology of dengue infection.

In conclusion, half the patients who presented with acute fever suspected of dengue infection during August to October were laboratory confirmed to have dengue infection in Nong Khai Province.

One death occurred (case fatality rate 1%). The majority of cases were among school age children. Dengue serotype 2 was the most prevalence serotype in the study. Only one case of chikungunya in a girl was identified. Since the disease varies by age and geographic location, increase awareness among health care workers and public health officers about these diseases in the area could help with early detection of cases and likely help with early prevention and control measures. Health education about these diseases and their prevention should be given to the public, especially in personal protection against mosquito bites.

ACKNOWLEDGEMENTS

We would like to thank the staff of Nong Khai, Phon Phisai, Phon Charoen Hospitals, Nongkhai Provincial Health Office, Arbovirus Laboratory and the National Institute of Health for their cooperation. We would also like to express our grateful to the MBDS staff for their support with this project. This study was funded by The Rockefeller Foundation through the Mekong Basin Disease Surveillance (MBDS) Thailand.

REFERENCES

Anantapreecha S, A-Nuegoonpipat A, Prakrong S, *et al*. Dengue virus cross-reactive hemagglutination inhibition antibody responses in patients with primary dengue virus infection. *Jpn J Infect Dis* 2007; 60: 267-70.

Bongsebandhu-phubhakdi C, Hemungkorn M, Thisyakorn U, Thisyakorn C. Risk factors influencing severity in pediatric dengue infection. *Asian Biomed* 2008; 2: 409-13.

Bureau of Epidemiology. Summaries of notifiable diseases: reported cases and deaths by provinces, Thailand, 2008 (2551). *Ann Epidemiol Surveill Rep* 2009; 1: 242-61.

Bureau of Epidemiology. Table of notifiable diseases: reported cases and deaths by provinces, Thailand, 2009 (2552). *Ann Epidemiol Surveill Rep* 2010; 1: 222-43.

Ditsuwan T, Liabsuetrakul T, Chongsuvivatwong V, Thammapalo S, McNeil E. Assessing the spreading patterns of dengue infection and chikungunya fever outbreaks in lower southern Thailand using a geographic information system. *Ann Epidemiol* 2011; 21: 253-61.

Faruque LI, Zaman RU, Alamgir AS, *et al*. Hospital-based prevalence of malaria and dengue in febrile patients in Bangladesh. *Am J Trop Med Hyg* 2012; 86: 58-64.

Heddini A, Janzon R, Linde A. Increased number of dengue cases in Swedish travellers to Thailand. *Euro Surveill* 2009; 14: 1-2.

Hochedez P, Jaureguiberry S, Debruyne M, *et al*. Chikungunya infection in travelers. *Emerg Infect Dis* 2006; 12: 1565-7.

Innis BL, Nisalak A, Nimmannitya S, *et al*. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989; 40: 418-27.

Nisalak A, Endy TP, Nimmannitya S, *et al*. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *Am J Trop Med Hyg* 2003; 68: 191-202.

Office of Diseases Prevention Control 6. K.M. Market & SRRT Seminar 2009. Khon Kaen: Office of Diseases Prevention Control 6, 2009.

Parida MM, Santhosh SR, Dash PK, *et al*. Rapid and real-time detection of chikungunya virus by reverse transcription loop-mediated isothermal amplification Assay. *J Clin Microbiol* 2007; 45: 351-7.

Pistone T, Ezzedine K, Boisvert M, *et al*. Cluster of chikungunya virus infection in travelers returning from Senegal, 2006. *J Travel Med* 2009; 16: 286-8.

Sa-ngasang A, Wibulwattanakij S, Chanama S,

et al. Evaluation of RT-PCR as a tool for diagnosis of secondary dengue virus infection. *Jpn J Infect Dis* 2003; 56: 205-9.

Sanohseang S, Ouppapong T. Summaries of selected notifiable diseases: dengue fever, dengue hemorrhagic fever, dengue shock syndrome. *Annu Epidemiol Surveill Rep* 2010; 1: 32-3.

Simon F, Parola P, Grandadam M, *et al*. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore)* 2007; 86: 123-37.

Strickman D, Sithiprasasna R, Kittayapong P, Innis BL. Distribution of dengue and Japanese encephalitis among children in rural and suburban Thai villages. *Am J Trop Med Hyg* 2000; 63: 27-35.

Suangtho P, Ouppapong T. Summaries of selected notifiable diseases: chikungunya fever. *Ann Epidemiol Surveill Rep* 2010; 1: 34-5.

Suttinont C, Losuwanaluk K, Niwatayakul K, *et al*. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. *Ann Trop Med Parasitol* 2006; 100: 363-70.

Tuntaprasart W, Barbazan P, Nitatpattana N, Rongsriyam Y, Yoksan S, Gonzalez JP. Seroepidemiological survey among schoolchildren during the 2000-2001 dengue outbreak of Ratchaburi Province, Thailand. *Southeast Asian J Trop Med Public Health* 2003; 34: 564-8.

Vallee J, Dubot-Peres A, Ounaphom P, Sayavong C, Bryant JE, Gonzalez JP. Spatial distribution and risk factors of dengue and Japanese encephalitis virus infection in urban settings: the case of Vientiane, Lao PDR. *Trop Med Int Health* 2009; 14: 1134-42.

World Health Organization (WHO). Guidelines for treatment of dengue fever/ dengue haemorrhagic fever in small hospitals. Geneva: WHO, 1999. [Cited 2013 Feb 18]. Available from: URL: http://www.searo.who.int/LinkFiles/Dengue_Guideline-dengue.pdf

World Health Organization (WHO) Regional Office for South-East Asia (WHO SEARO). Prevention and control of dengue and dengue haemorrhagic fever : comprehensive guidelines. New Delhi: WHO SEARO, 1999.

Yenchitsomanus PT, Sricharoen P, Jaruthasana I, *et al*. Rapid detection and identification of dengue viruses by polymerase chain reaction (PCR). *Southeast Asian J Trop Med Public Health* 1996; 27: 228-36.