International Journal of Infectious Diseases 14 (2010) e888–e892

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Japanese encephalitis virus remains an important cause of encephalitis in Thailand

Sonja J. Olsen [a,*], Krongkaew Supawat [b], Angela P. Campbell [c,d], Surapee Anantapreecha [b], Sahas Liamsuwan [e], Supoch Tunlayadechanont [f], Anannit Visudtibhan [f], Somsak Lupthikulthum [g], Kanlaya Dhiravibulya [h], Akravudh Viriyavejakul [h], Punnee Vasiknanonte [i], Kiatsak Rajborirug [j], Veerachai Watanaveeradej [k], Chacrin Nabangchang [k], Janeen Laven [l], Olga Kosoy [l], Amanda Panella [l], Christine Ellis [l], Sununta Henchaichon [m], Nino Khetsuriani [c], Ann M. Powers [l], Scott F. Dowell [n], Marc Fischer [l]

[a] Divison of Emerging Infections and Surveillance Services, Centers for Disease Control and Prevention Collaboration, 1600 Clifton Road, MS C-12, Atlanta, GA 30333, USA
[b] National Institutes of Health, Ministry of Public Health, Nonthaburi, Thailand
[c] Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[d] Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[e] Queen Sirikit National Institute of Child Health, Bangkok, Thailand
[f] Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[g] Rajvithi Hospital, Bangkok, Thailand
[h] Prasat Neurological Institute, Bangkok, Thailand
[i] Prince Songkhla University Hospital, Hat Yai, Thailand
[j] Hat Yai Hospital, Hat Yai, Thailand
[k] Phramongkutklao Hospital, Bangkok, Thailand
[l] Division of Vector Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA
[m] International Emerging Infections Program, Thailand Ministry of Public Health – US CDC Collaboration, Nonthaburi, Thailand
[n] Coordinating Office for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

ARTICLE INFO

Article history:
Received 8 January 2010
Accepted 11 March 2010

Corresponding Editor: William Cameron, Ottawa, Canada

Keywords:
Japanese encephalitis
Encephalitis
Surveillance
Vaccine

SUMMARY

Background: Japanese encephalitis virus (JEV) is endemic in Thailand and prevention strategies include vaccination, vector control, and health education.
Methods: Between July 2003 and August 2005, we conducted hospital-based surveillance for encephalitis at seven hospitals in Bangkok and Hat Yai. Serum and cerebrospinal (CSF) specimens were tested for evidence of recent JEV infection by immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) and a plaque reduction neutralization test (PRNT).
Results: Of the 147 patients enrolled and tested, 24 (16%) had evidence of acute flavivirus infection: 22 (15%) with JEV and two (1%) with dengue virus. Of the 22 Japanese encephalitis (JE) cases, 10 (46%) were aged ≤15 years. The median length of hospital stay was 13 days; one 13-year-old child died. Ten percent of encephalitis patients enrolled in Bangkok hospitals were found to have JEV infection compared to 28% of patients enrolled in hospitals in southern Thailand ($p < 0.01$). Four (40%) of the 10 children with JE were reported as being vaccinated.
Conclusions: JEV remains an important cause of encephalitis among hospitalized patients in Thailand. The high proportion of JE among encephalitis cases is concerning and additional public health prevention efforts or expanded vaccination may be needed.
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1. Introduction

Japanese encephalitis virus (JEV), a mosquito-borne virus, is an important cause of encephalitis in Asia with an estimated 50 000 cases of Japanese encephalitis (JE) and 10 000 deaths each year. JE is a severe disease and up to 50% of persons who survive it can have prolonged neurological or psychiatric sequelae.[1] JE is endemic in Thailand, with between 1500 and 2500 cases reported annually throughout the 1970 s and 1980 s.[2,3]

To reduce the burden of disease, in 1973, the Thailand Ministry of Public Health (MOPH) implemented a JE control program that initially focused on vector control. In 1990, a locally produced

* Corresponding author. Tel.: +1 404 639 7883; fax: +1 404 639 3106.
E-mail address: SOlsen@cdc.gov (S.J. Olsen).

1201-9712/$36.00 – see front matter. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.
doi:10.1016/j.ijid.2010.03.022

inactivated mouse brain-derived JEV vaccine was introduced into the routine immunization schedule.[4] Children aged 1 to 3 years receive two primary doses four weeks apart and then a booster after one year.[5] By the mid-1990 s, the JE control program was multi-faceted and included education campaigns, vector control, and vaccination.[3] In 2003, a vaccination coverage survey conducted in 12 provinces found that among children 2 to 3 years of age, 92% and 87% had received their first and second dose of JE vaccine, respectively (Thailand MOPH, unpublished data). In 3- to 4-year-olds, the survey found 89%, 85%, and 62% coverage for doses one, two, and three, respectively.

As a result of these interventions, over the past 20 years Thailand has achieved dramatic reductions in the number of JE cases reported nationally.[6] Between 2002 and 2008, the number of encephalitis cases reported nationally ranged from 297 to 418 per year, representing a four- to eight-fold decrease from earlier decades.[7]

In 2003, we conducted a rigorous prospective assessment of the frequency of JEV as a cause of encephalitis in a country with good JEV vaccination coverage. Our findings suggest that JEV remains a relatively common cause of encephalitis in the two areas of Thailand studied.

2. Methods

2.1. Case enrollment

Patients were identified from all wards at five hospitals in Bangkok (Queen Sirikit National Institute of Child Health, Rajvithi Hospital, Ramathibodi Hospital, Prasat Neurological Institute, Phramongkutklao Hospital) and two in Hat Yai (Hat Yai Hospital and Prince Songkhla University Hospital) between July 2003 and August 2005. Inpatients meeting the following criteria were approached for enrollment: (1) fever or hypothermia (i.e., history of fever or documented temperature $\geq$38 °C or $\leq$35 °C); (2) evidence of acute brain dysfunction (i.e., encephalopathy, central neurological findings, or seizures) with onset $\leq$14 days prior to admission; and (3) clinical indication for lumbar puncture as determined by a staff physician. Eligible patients or their parent/guardian were asked to provide informed consent.

For each consenting patient, the attending neurologist and a research nurse used standardized forms to collect the following information: demographics, clinical symptoms, results of imaging studies, treatment prior to enrollment, exposures one month before the onset, travel history, vaccination history, and significant medical/social history. In addition, the nurse recorded relevant laboratory data obtained as part of the patient's clinical care. Cerebrospinal fluid (CSF), saliva, oropharyngeal swab, urine, stool, whole blood, and acute serum samples were collected from each patient at admission. Patients who had been discharged were asked to return for a convalescent visit 3 to 5 weeks after admission.

A case of encephalitis was defined as a patient who met the enrollment criteria and had neuroimaging (i.e., computed tomography (CT) or magnetic resonance imaging (MRI)) or electroencephalography (EEG) findings consistent with encephalitis, or CSF pleocytosis (white blood cell (WBC) counts: $\leq$6 weeks old, $>14 \times 10^6$/l; >6 weeks old, $>4 \times 10^6$/l).

The protocol was approved by the ethics committee of each participating hospital, the Ethics Review Committee of the Thailand Ministry of Public Health, and the Institutional Review Board of the US Centers for Disease Control and Prevention (CDC).

2.2. Laboratory testing

Specimens were split prior to freezing and tested both at CDC and the Thai National Institutes of Health (NIH). Serum and CSF samples from patients enrolled in the study were tested at the Arboviral Diseases Branch, CDC, Fort Collins, CO, USA. Specimens were tested using JEV and dengue virus (DENV) immunoglobulin M (IgM) capture enzyme-linked immunosorbent assays (ELISA).[8] Specimens testing positive for JEV or DENV IgM antibodies in serum or CSF were tested for JEV and DENV neutralizing antibodies by plaque reduction neutralization tests using a 90% cut-off value ($PRNT_{90}$).[9] For patients with serological evidence of recent DENV infection, acute serum or CSF samples (i.e., collected $\leq$7 days after symptom onset) were also evaluated using a reverse transcription polymerase chain reaction (RT-PCR) for DENV RNA.[10] The Thai NIH conducted IgM ELISA for JEV on serum and CSF.[11] These results were compared to CDC laboratory results to evaluate in-country diagnostics.

2.3. Case classification

For the purpose of describing the epidemiology of hospitalized patients with JEV infection, case status was determined based on the CDC laboratory results. A confirmed case of recent JEV infection was defined as the presence of (1) JEV IgM antibodies in CSF, or (2) JEV IgM antibodies in serum with a JEV $PRNT_{90}$ titer $\geq$20 and a JEV $PRNT_{90}$ to DENV $PRNT_{90}$ titer ratio $\geq$4. A probable case had JEV IgM antibodies in serum with a JEV $PRNT_{90}$ titer $\geq$20 but the JEV $PRNT_{90}$ to DENV $PRNT_{90}$ titer ratio was $<$ 4. A non-JE case was defined as a patient who met the case definition for encephalitis and had a serum or CSF specimen collected 10 or more days after fever onset that tested negative at CDC for JEV IgM antibodies. Patients with DENV infection were excluded from this non-JE case classification.

A confirmed case of recent DENV infection was defined as the presence of (1) DENV RNA in serum or CSF, or (2) DENV IgM antibodies in serum with a DENV $PRNT_{90}$ titer $\geq$20 and a DENV $PRNT_{90}$ to JEV $PRNT_{90}$ titer ratio $\geq$4.

2.4. Statistical analysis

Frequencies were compared using Chi-square for dichotomous variables and analysis of variance (ANOVA) for continuous variables. A p-value of $<$0.05 was considered significant. All analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, USA). For computing test sensitivity and specificity, we used the CDC assay as the gold standard. Results from the Thai NIH were classified as JEV infection, not JEV infection, or not interpretable. For the purposes of the comparison of results between the two laboratories, we considered those marked by the Thai NIH as not interpretable to be negative.

3. Results

Between July 2003 and August 2005, 150 hospitalized patients met the case definition for acute encephalitis. Of these, 147 (98%) had specimens available for testing at CDC. Evidence of recent flavivirus infection was identified in 24 (16%) patients: 22 with JEV (16 confirmed and six probable) and two with dengue (both confirmed). The proportion of encephalitis cases due to JEV varied by hospital: 6/21 (29%) in Hat Yai, 5/18 (28%) in Prince Songkhla, 4/15 (27%) in Prasat Neurological Institute, 1/6 (17%) in Rajvithi, 1/11 (9%) in Phramongkutklao, 3/36 (8%) in Ramathibodi, and 2/40 (5%) in Queen Sirikit National Institute of Child Health. Ten percent of encephalitis patients enrolled in Bangkok hospitals were found to have JEV infection compared to 28% of patients enrolled in hospitals in southern Thailand ($p < 0.01$). There were 107 patients who had adequate testing to rule out JEV infection and who were classified as non-JE cases. The remaining 16 patients did not have adequate specimens to either rule in or rule out JEV infection.

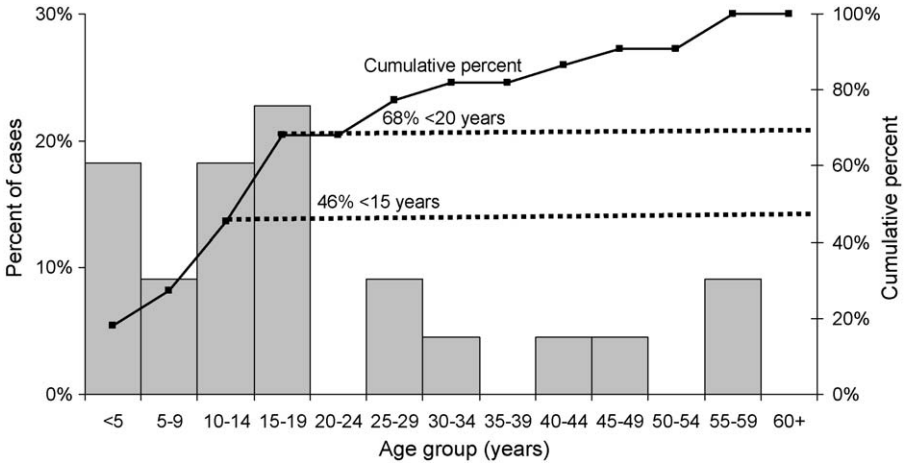

Fig. 1. Percent and cumulative percent of patients with Japanese encephalitis virus by age group.

3.1. Japanese encephalitis

3.1.1. Basic demographics

Of the 22 cases with JEV infection, 14 (64%) were male and the median age was 19 years (range 6 months to 57 years). Overall, 10 (46%) of the JE patients were aged <15 years and another five (23%) patients were aged 15–19 years (Figure 1). Ten (46%) were reported from the hospitals in southern Thailand and 12 (54%) from the hospitals in Bangkok. Patients with JEV infection were identified throughout the year, with a peak from March through June (Figure 2). The numbers were too small to assess seasonal variability by site and year. Fourteen (64%) patients with JEV infection reported living in a rural area (farm or countryside) in the past three months; two patients reported residing in Bangkok Province when illness began. Patients with confirmed and probable JEV infection were similar with respect to age (19 years vs. 24 years, $p = 0.6$) and male sex (69% vs. 50%, $p = 0.6$).

3.1.2. Clinical characteristics and outcomes

Among the 22 patients with JEV infection, 21 (95%) had altered mental status, including seven (32%) who were comatose. Fifteen (68%) patients had stiff neck, and 10 (45%) had seizures, including eight (80%) of the 10 children aged <15 years. Of the 22 JE patients, 21 (95%) had CSF pleocytosis; 11 (50%), including the one patient without pleocytosis, had abnormal neuroimaging, and three (14%) had an abnormal EEG. The median CSF WBC count was 66×10^6/l (range 0–690×10^6/l), protein was 70 mg/dl (range 20–218 mg/dl), and glucose was 62 mg/dl (range 1–138 mg/dl). Of all 22 patients, seven (32%) had an elevated CSF opening pressure (>60 mm for children and >200 mm for adults). The median length of hospital stay was 13 days (range 4–173 days). One (5%) JE patient, aged 13 years, died. Of the 21 surviving patients, nine were seen after hospital discharge and one (11%) reported poor cognitive recovery.

JE patients were significantly more likely to present with stiff neck or focal neurological signs compared to other encephalitis patients without JE (Table 1). No other differences were identified in patient demographics, clinical characteristics, or outcomes.

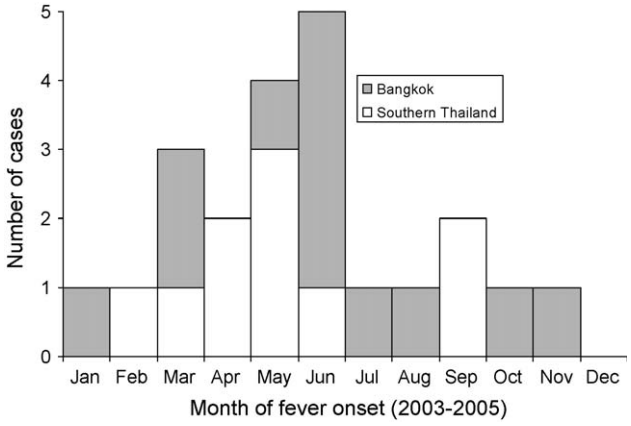

Fig. 2. Seasonality of Japanese encephalitis in patients by site of enrollment (Bangkok versus southern Thailand), July 2003 through August 2005.

3.1.3. Vaccination history

Four (18%) JE patients reported having received JE vaccine. Two children, both 2 years of age, had each received two doses of JE vaccine with the last dose administered approximately one year prior to their illness. A 6-year-old had received one dose of JE vaccine almost five years before illness onset. An 18-year-old also reported being vaccinated but no dates were given. Of the remaining JE patients, 12 (54%) had not received JE vaccine and six (27%) did not know their vaccination status. Given that country-wide childhood vaccination began in 2000, two of the 12 unvaccinated persons (a 3-year-old and a 7-year-old) were likely eligible for JE vaccine as part of the national program between 2000 and 2005.

3.1.4. Laboratory comparison of JE testing

Of the 147 encephalitis patients tested at CDC, 146 (99%) had specimens available for testing at the Thai NIH. Of the 22 JEV infections identified at CDC, 21 (95%) were also identified as recent JEV infections by the Thai NIH. Of the 124 JEV-negative infections, all were negative or not interpretable at the Thai NIH.

3.2. Dengue fever

Two patients meeting the encephalitis case definition had evidence of recent DENV infection. The first was a 4-year-old girl who presented with a temperature of 39.6 °C and CSF with a WBC count of 0×10^6/l, 31 mg/dl protein, and 216 mg/dl glucose and a Glasgow coma score of 4. The patient experienced seizures and focal neurological signs, and presented to hospital in a coma. No further information was available to suggest evidence of hemorrhage or shock. An EEG was abnormal and demonstrated asymmetrical features; neuroimaging was not done. Acute and convalescent sera were collected at 5 days and 30 days after illness onset. DENV IgM antibodies were identified in both specimens and

Table 1
Comparison of characteristics of patients with encephalitis with and without laboratory-confirmed Japanese encephalitis

Characteristic	Patients with Japanese encephalitis ($N=22$), n (%)	Patients without Japanese encephalitis ($N=107$), n (%)	p-Value
Demographic			
Male	14 (64)	57 (53)	0.37
Median age (range)	19 years (0–57)	11 years (0–83)	0.81
Currently live in rural area[c]	14 (64)	51 (48)	0.19
Clinical			
Altered mental status	21 (95)	86 (80)	0.07
Stiff neck	15 (68)	38 (36)	<0.01[a]
Headache	14 (64)	50 (47)	0.22
Personality change	12 (55)	40 (37)	0.05
Focal neurological signs	11 (50)	33 (31)	0.01[a]
Seizure	10 (45)	49 (46)	0.97
Coma	7 (32)	12 (11)	0.05
Median CSF WBC (range)[b]	66×10^6/l (0–690)	22×10^6/l (0–4150)	0.83
Outcome			
Median length of hospital stay (range)	13 days (4–173)	18 days (2–180)	0.17
Died	1 (5)	4 (4)	0.86
Exposure			
Mosquito bite	19 (86)	95 (89)	0.75
JE vaccine	4[d] (18)	39[e] (36)	0.08

CSF, cerebrospinal fluid; WBC, white blood cell count; JE, Japanese encephalitis.
[a] Current defined as in the past 3 months.
[b] One patient with JE and two patients without JE were missing CSF WBC.
[c] Significant at $p < 0.05$.
[d] Two persons had one dose; two persons had two doses.
[e] Thirty-five persons had two or more doses.

DENV neutralizing antibody titers increased 16-fold between the acute and convalescent serum. CSF was not available for DENV antibody testing and there was no remaining serum for DENV RT-PCR. The second patient was a 12-year-old boy who presented with a temperature of 39.0 °C and CSF with a WBC count of 7×10^6/l, 25 mg/dl protein, and 89 mg/dl glucose and a Glasgow coma score of 15. Before hospitalization, the patient experienced personality change. EEG was normal and neuroimaging was not done. Acute and convalescent sera were drawn at 3 days and 33 days after illness onset. DENV IgM antibodies were identified in both specimens and DENV neutralizing antibody titers increased four-fold between the acute and convalescent serum. The acute serum was positive by RT-PCR for DENV RNA. CSF was not available for DENV antibody or RT-PCR testing.

4. Discussion

JEV remains an important cause of encephalitis in Thailand. Although vector control efforts and vaccine introduction in 2000 have resulted in a reduced disease burden, the data presented here suggest that JE continues to be a public health concern, causing an estimated 15% of hospitalized encephalitis cases, and affecting not only children but also adolescents and adults.

Clinical differentiation of the causes of encephalitis can be challenging, and JE presents like encephalitis of many other causes. In this study, patients with JE were significantly more likely to present with stiff neck or focal neurological signs compared to patients with encephalitis not due to JEV. These findings might help guide clinicians in the diagnosis.

Interestingly, in this study of hospitalized patients, the proportion of encephalitis patients with JEV infection was significantly higher among the southern hospitals than those in Bangkok. This difference cannot be wholly explained by vaccine introduction because it was introduced the same year, 2000, in Bangkok and suburbs, as well as the southern provinces. It may be that civil unrest in southern Thailand contributed to lower vaccination uptake in the population, although vaccine coverage surveys in a nearby province do not support that hypothesis. The difference may instead reflect other factors, such as ecological variability in JEV or vector density between areas, or relative differences in other more prevalent causes of encephalitis.

Similar to recent studies in Nepal demonstrating a large proportion of JE in adults,[12,13] 55% of our patients with JE were aged 15 years or older. In Thailand, nationwide JE vaccination began in 2000, and JE vaccine coverage studies conducted in 2003 showed high coverage of the first two doses. These studies included two provinces neighboring our study sites: Nonthaburi which borders Bangkok and Phatthalung which borders Songkhla. In 2006, only persons between the ages of 1 and 6 years should have been vaccinated. There were five patients with JEV in this study who should have been vaccinated and three of them were, although none received all three doses of the vaccine. The majority of patients in this study who developed JEV infection were over 6 years old and therefore not vaccinated. Additional data, such as incidence to determine the burden of JEV disease in Thailand, may help to determine whether additional strategies, such as a catch-up campaign to vaccinate adolescents and adults, should be considered to reduce disease in adults.

There were two patients who had convincing laboratory evidence of recent dengue infection, but neither had convincing evidence of central nervous system inflammation. Although the role of DENV in encephalitis is still controversial, a few studies have documented neurological findings.[14,15] In locations like Thailand where JEV and DENV are endemic, it is prudent to test specimens for both viruses.

Historically, JE is thought to be a disease largely transmitted in rural areas and our findings support that claim; the majority of patients reported living in a rural area in the three months before illness. However, there is increased interest in the importance of urban or peri-urban environments, where large populations with high density reside. A study by Gingrich and colleagues in suburban Bangkok found that transmission can be sustained if conditions are right.[16] In our study, only two patients with JEV infection were reportedly in Bangkok proper, although it is impossible to know precisely where transmission occurred.

Although Thailand's strategy to combat JEV is multifaceted, a core component is vaccination using a locally produced, affordable vaccine. With the development of new JEV vaccines, such as the

live-attenuated SA 14–14–2 vaccine, and a World Health Organization recommendation to gradually replace the mouse-brain derived vaccine with new vaccines, Thailand may reconsider the current strategy.[5,17] Human vaccination will continue to be central to any prevention efforts since the complexity of controlling JEV transmission cycles makes elimination unlikely.[18]

Acknowledgements

The authors thank Tawee Chotpitayasunondh, Khanchit Limpakarnjanarat, Samart Karuchit, Robert Lanciotti, and Henry 'Kip' Baggett for their technical support. The authors also thank Susan Hills for helpful comments on the manuscript.

Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Funding source: This research was supported by the Centers for Disease Control and Prevention.

Ethical approval: The protocol was approved by the ethics committee of each participating hospital, the Ethics Review Committee of the Thailand Ministry of Public Health, and the Institutional Review Board of the US Centers for Disease Control and Prevention (CDC).

Conflict of interest: We have no competing interests to declare.

References

1. Fischer M, Hills S, Staples E, Johnson B, Yaich M, Solomon T. Japanese encephalitis prevention and control: advances, challenges, and new initiatives. In: Scheld WM, Hammer SM, Hughes JM, editors. *Emerging infections 8*. Washington, DC: ASM Press; 2008. p. 93–124.
2. Chunsuttiwat S. Japanese encephalitis in Thailand. *Southeast Asian J Trop Med Public Health* 1989;**20**:593–7.
3. Chunsuttiwat S, Warachit P. Japanese encephalitis in Thailand. *Southeast Asian J Trop Med Public Health* 1995;**26**(Suppl 3):43–6.
4. Pongpairoj S, Choakouychai B, Boonrueng C, Kutirakan P, Ahandrik S, Leelasiri K, et al. A test production of inactivated mouse brain JE vaccine in Thailand. *Southeast Asian J Trop Med Public Health* 1989;**20**:647–52.
5. World Health Organization. Japanese encephalitis vaccines. Wkly Epidemiol Rec 2006; 81:331–40.
6. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13–15 October 1998. *Vaccine* 2000;**18**(Suppl 2):1–25.
7. Thailand Ministry of Public Health. Annual epidemiologic surveillance reports, 2002-2008. Available at: http://203.157.15.4/Annual/Total_Annual.html. (accessed 8 September, 2009).
8. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;**38**:1823–6.
9. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *J Gen Virol* 1989;**70**(Pt 1):37–43.
10. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 1992;**30**:545–51.
11. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn 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.
12. Partridge J, Ghimire P, Sedai T, Bista MB, Banerjee M. Endemic Japanese encephalitis in the Kathmandu valley. *Nepal Am J Trop Med Hyg* 2007;**77**:1146–9.
13. Wierzba TF, Ghimire P, Malla S, Banerjee MK, Shrestha S, Khanal B, et al. Laboratory-based Japanese encephalitis surveillance in Nepal and the implications for a national immunization strategy. *Am J Trop Med Hyg* 2008;**78**:1002–6.
14. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, et al. Neurological manifestations of dengue infection. *Lancet* 2000;**355**:1053–9.
15. Misra UK, Kalita J, Syam UK, Dhole TN. Neurological manifestations of dengue virus infection. *J Neurol Sci* 2006;**244**:117–22.
16. Gingrich JB, Nisalak A, Latendresse JR, Sattabongkot J, Hoke CH, Pomsdhit J, et al. Japanese encephalitis virus in Bangkok: factors influencing vector infections in three suburban communities. *J Med Entomol* 1992;**29**:436–44.
17. World Health Organization. Global Advisory Committee on Vaccine Safety, 9-10 June 2005. Wkly Epidemiol Rec 2005; 80:242-7.
18. Solomon T. Control of Japanese encephalitis—within our grasp? *N Engl J Med* 2006;**355**:869–71.