JAPANESE ENCEPHALITIS IN MALAYSIA: REVIEW OF LABORATORY DATA FROM 2006 TO 2013

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Abstract. Between 2006 and 2013, a total of 3,614 clinical specimens were received from various hospitals in Malaysia at the Institute for Medical Research, from patients with clinically suspected viral encephalitis. These specimens, comprising of 1,861 sera and 1,753 cerebrospinal fluid (CSF), were tested for JEV IgM antibodies. The incidence of laboratory confirmed JE, with positive JEV IgM was 4.84%. No annually significant decrease was observed in the incidence of laboratory confirmed JE in the years studied and children aged 5-15 years old showed the highest incidence rate encompassing 44.38% (75/169) of all positive cases.

Keywords: Japanese encephalitis, JEV IgM antibodies, viral encephalitis, Malaysia

INTRODUCTION

Japanese encephalitis (JE) is an infection caused by the arthropod-borne flavivirus, JE virus (JEV). JEV is a single stranded, positive sense, enveloped icosahedral virus of about 50nm diameter. The main reservoir and amplifying hosts are pigs, with water birds and mosquitoes as carriers and vectors, respectively (Unni, 2011). Due to low viremic levels and short term viremia, humans are known as dead end hosts. JE is known as a major cause of viral encephalitis in the endemic regions of Asia and the Pacific (Campbell *et al*, 2011). The primary vector of the virus is the Culicine mosquitoes, namely *C. tritaeniorhynchus*, *C. vishnui*, *Culex gelidus* and *C. fascocephala*, which usually breed in the rice fields (Thongcharoen, 1989). Transmission is higher in areas with coexisting rice farming and pig-rearing practices and in Malaysia, sporadic cases occur year round with peaks during the rainy season (Erlanger *et al*, 2009).

The first reported case of JE in the world was in Japan in 1871, and the first confirmed case of Japanese encephalitis in Malaysia was reported by Paterson *et al* in 1952. In Malaysia, outbreaks of JE have been reported in Pulau Langkawi (1974), Penang (1998), and Serian, Sarawak in 1992 (Lam, 1999).

Laboratory diagnosis of JE infection is done by viral isolation, serology and molecular detection. WHO recommends the antibody detection method of JEV IgM capture enzyme linked immunosorbent assay (MAC ELISA) test which was a less time consuming method as a laboratory confirmation test for JE.

JE MAC ELISA test was introduced in 1993 in the Virology Unit, Institute for Medical Research (IMR), Kuala Lumpur. However, the test has since then been

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replaced by Panbio JE-Dengue IgM Combo ELISA from 2011. The unit has been involved in the laboratory diagnosis of Japanese encephalitis cases from all over Malaysia and was designated as the WHO National Laboratory for JE from 2011 to 2012. The objective of this study was to review laboratory data to determine the incidence of laboratory confirmed Japanese encephalitis in Malaysia from 2006 to 2013.

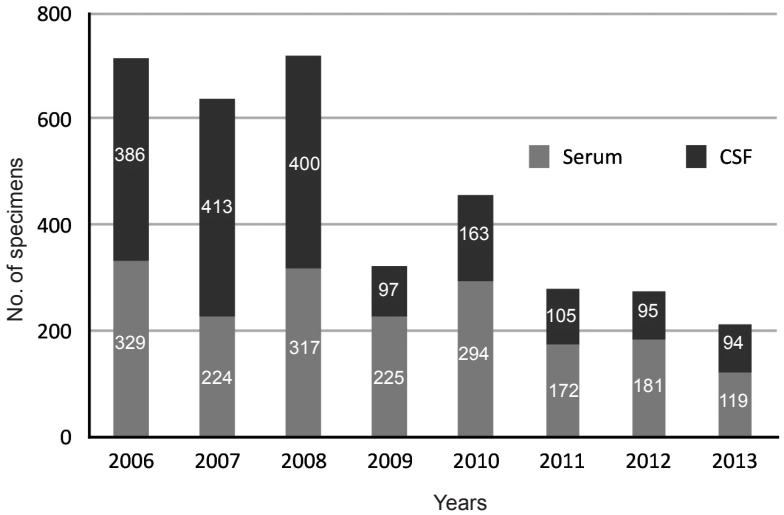

Fig 1–Number of specimens tested for JEV IgM from 2006 to 2013.

MATERIALS AND METHODS

Samples

Between 2006 to 2013 a total of 3,614 specimens, sera and cerebrospinal fluid (CSF) samples, were received at IMR for laboratory diagnosis of suspected viral encephalitis. All of the 3,614 samples received were screened for presence of JEV IgM specific antibodies.

Ethical clearance

Specimens were collected as part of routine laboratory diagnosis and the study used retrospective data only and not human subjects. The analysis used only deintentified and aggregate laboratory data. Therefore this study did not require ethics review.

JEV IgM assay

The samples received from 2006 till 2010 were tested for JEV specific IgM by the JE MAC ELISA IgM method as described by Lam *et al* in 1987. Specific JE antigen was procured from National Institutes of Health, Nonthaburi, Thailand.

Samples received from 2011 to 2013 were tested by using Panbio JE-Dengue IgM Combo ELISA in which test procedures, interpretation and validation of test results were conducted following the manufacturer's instruction (Inverness Medical Innovations Australia, Queensland, Australia).

Data analysis

Data was analysed using 2-way ANOVA in GraphPad Prism 5 for Windows version 5.01. A p-value < 0.05 was considered statistically significant.

RESULTS

The total number of clinical specimens received from various hospitals in Malaysia from 2006 to 2013 was 3,614. The specimens comprised of 1,861 sera and specimens 1,753 CSF (Fig 1). JEV IgM antibody was detected in 175 specimens, from which 121 were serum and 54 were CSF specimens. Some patients had both CSF and serum specimens sent, while others only sent a single specimen of serum or CSF.

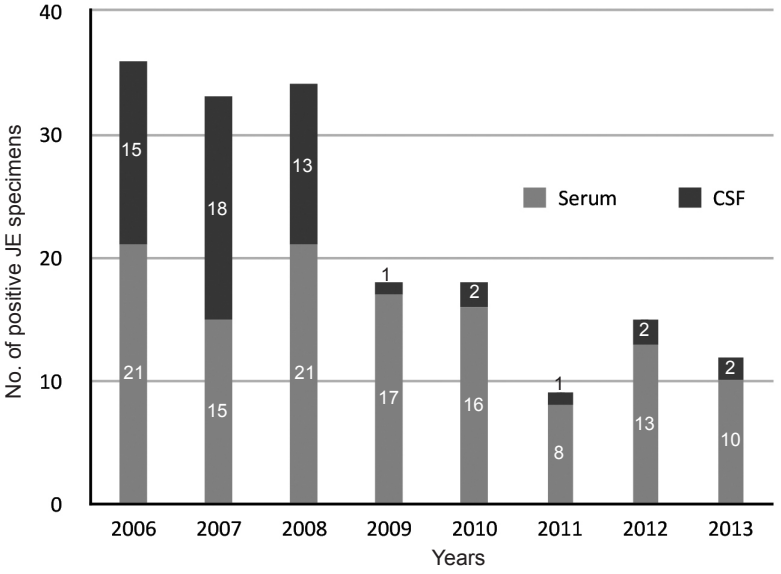

Fig 2–Distribution of laboratory confirmed JE specimens from 2006 to 2013.

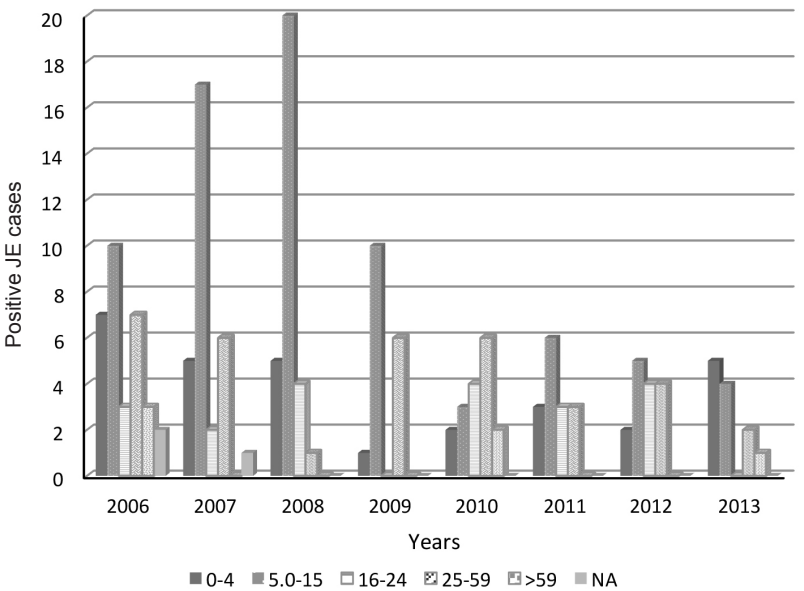

Fig 3–Age distribution of laboratory confirmed JE cases from 2006 to 2013.

In this study, the positivity of the JEV IgM assay from laboratory specimens received at IMR for the years from 2006 to 2013 was 4.84% (Fig 2). The incidence of positive JEV IgM by year were 5.03% (36/715) in 2006, 5.18% (33/637) in 2007, 4.74% (34/717) in 2008, 5.59% (18/322) in 2009, 3.94% (18/457) in 2010, 3.24% (9/277) in 2011, 5.43% (15/276) in 2012 and 5.63% (12/213) in 2013. No significant difference was observed ($p > 0.05$) in the incidence of confirmed JE from the years of 2006 to 2013. However, there is a significant decrease in the number of positive cases for CSF samples in 2009 and 2013 [$p < 0.05$, 2009: 95% Confidence interval (CI) -12.64to-0.3865; 2013: 95% CI -6.27 to -0.1265].

Children aged 5-15 years old shows the highest incidence rate (Fig 3). They encompasses 44.38% (75/169) of all positive cases. The states in Malaysia with the highest incidence rate was Selangor with 15.98%, (27/169), with the highest number of cases in the year 2007, followed by Perak with15.38% (26/169) of the total infection rate and with the highest number of cases detected in 2006.

There was no significant difference ($p > 0.05$) in incidence detected between male and female patients with positive JE results (Fig 4).

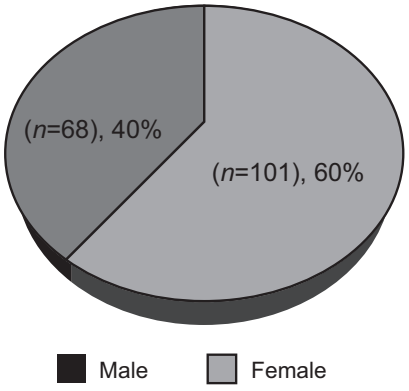

Fig 4–JE cases by gender from positive JEV IgM specimens 2006 to 2013.

DISCUSSION

Laboratory diagnosis of Japanese encephalitis is crucial for accurate diagnosis and surveillance. The virus is difficult to isolate from clinical specimens because of low viremia and rapid development of virus neutralizing antibodies. The detection of JEV specific IgM has been accepted as the standard serological diagnosis for JEV. In the present study, we analysed the anti JEV IgM positive specimens received in the laboratory and found that less than 5% of specimens received from suspected acute viral encephalitis cases were caused by Japanese encephalitis infection. A similar study in 1996 by Balasubramaniam *et al*, had shown that between 1993 and 1995 the overall incidence of the JE infection was 7.6%. Our rates are lower compared to Thailand (Olsen *et al*, 2010) which showed 10-28% of encephalitis patients were found to have JE virus (JEV) infection, and Vietnam (Thu Yen *et al*, 2010) showing 52% of the acute encephalitis syndrome (AES) cases were laboratory confirmed as JEV infection.

The number of specimens received in this study had decreased considerably from 715 specimens in 2006 to only 213 specimens in 2013. A significant decrease in the number of positive cases for CSF samples in 2009 and 2013 was also observed.This data likely follows the reduction in the national incidence rate of viral encephalitis from 0.09 per 100,000 population in 2006 to 0.04 per 100,000 population in 2011 (Malaysia Ministry of Health, Health Facts 2006 and 2012).

Campbell *et al* in 2011 estimated an annual occurrence of about 67,900 JE cases in JE-endemic countries with an incidence of 1.8 per 100,000 in children 14 years and younger. However, his estimated incidence for Malaysia was 4.7 per 100,000 for Sarawak and 5.3 per 100,000 for Peninsular Malaysia. Sarawak had included JE vaccination in their Extended Programme of Immunization (EPI) since 2001. Wong *et al* (2008) reported a reduced incidence of JE infection since the introduction of the Biken JE vaccine in Sarawak in 2001 from 9.8 per 100,000 population under 12 years of age to 4.3 per 100,000 population under 12 years in 2006. In comparison, our neighboring country Thailand had started introducing the JE vaccination program in 1990 and expanded it into nationwide EPI in 2000 (Olsen *et al*, 2010), and reported a reduction of 4-8 fold decrease between 2002-2008 compared to 10 years earlier. Vietnam had introduced JE vaccination program in 1997 in high risk districts, followed by plans of expansion into national EPI program by 2011. The country's JE incidence had decreased to 1.4 cases per 100,000 population from 1 to 8 cases per 100,000 in 1985-1993 (Yen *et al*, 2010). These countries had demonstrated success in the reduction of JEV infection through the introduction of JE vaccination into the national EPI.

The most reliable method for JE diagnosis is by detection of IgM antibody in CSF, as the presence of the IgM antibody

indicates a CNS infection (Hills *et al*, 2009). However, if CSF is unavailable, the detection of JEV IgM in serum can confirm a JE infection. Second samples or paired sera samples were usually requested for acute/convalescent phase to detect JE infection as JEV IgM antibody might not be detectable in early, acute single specimen. However, in this study, it is quite rare to receive repeat samples from patients, therefore likely affecting the true rate of JE infection. This is especially in patients in early phase of infection as only about 70-75% of patients have detectable serum IgM in specimens collected up to 4 days of onset (WHO, 2007).

In endemic regions, JE is mainly a disease in the rural areas and infecting primarily children less than 15 years old (Hills *et al*, 2009). Its incubation period varies between 5 to 15 days and clinical symptoms ranges from mild acute febrile illness to neurological involvement causing seizures, meningitis, encephalitis and poliomyelitis-like symptoms. Case fatality rate is between 20-40% and neurological sequelae occurs in up to 50% of patients (Ooi *et al*, 2008). In this study, the data showed the highest incidence rate is in children population aged from 5 to 15 years old, which correlates with literature studies.

In endemic areas where JE vaccine is incorporated into the EPI for 1-2 years old children, a shift in the incidence in older children is a concern. Therefore, 'catch-up' vaccination is a consideration in these cases. In Malaysia, a hospital-based surveillance study for Japanese encephalitis carried out during 1997 - 2006 in Sarawak by Wong *et al* (2008) found the introduction of the JE vaccine had reduced the incidence of JE infection in children but increased the mean age of infected children from 6.3 to 8 years old. Therefore, in these cases, the older age group and elderly are more exposed to the JE virus infection. This presentation was demonstrated in studies from Thailand and Nepal with a high proportion of patients aged more than 15 years old having JEV infection (Wierzba *et al*, 2008; Olsen *et al*, 2010).

Surveillance of Japanese encephalitis infection is important in the prevention and control of the disease. WHO recommends clinical syndromic surveillance for AES, as JE is usually clinically indistinguishable from other encephalitis causes. This should be followed by laboratory confirmation of JE virus infection by MAC ELISA for JE.

Currently, JE vaccine has not been included in the EPI in Peninsular Malaysia. It is unclear whether there is a need to consider inclusion of JE vaccine in the current EPI based on the fact that JE is also endemic in peninsular Malaysia. Therefore, this emphasises the importance of close monitoring and sero-surveillance studies in the country. The activity would yield the actual prevalence of the infection in the country and therefore allow the determination of disease burden and trends of the disease. This data could in turn be used in the determination of the feasibility and cost-effectiveness of any control and prevention activities such as vector control or immunizations.

Pigs are known as major amplifying hosts of Japanese encephalitis virus and in endemic areas could also act as maintenance hosts (van den Hurk *et al*, 2009). As mentioned earlier, JE vaccination is included in EPI in Sarawak, but in Peninsular Malaysia JE vaccination is only administered to pig farm workers. However, when there is an outbreak, vaccination will also be given to residents living within 2 km radius from the pig farm. Several states in Peninsular Malay-

sia are known to have pig-rearing farms. Whether this impacted on the incidence rate of JE infection in Peninsular Malaysia has not been studied. However, based on our data, Selangor and Perak having pig rearing farms were the two states with the highest incidence rate.

Our study has not shown any significant difference between genders in relation to JEV infection. However, there are other studies that demonstrated different rates of JEV infection in men compared to women (Akiba *et al,* 2001; Partridge *et al*, 2007).

In conclusion, our findings demonstrated that less than 5% of specimens from patients diagnosed clinically as viral encephalitis cases have detectable serum JEV IgM. Reduction in number of suspected viral encephalitis samples sent to our laboratory in a span of 8 years was also observed. This study also indicated similar results in literatures that observed the highest prevalence for Japanese encephalitis virus infection in pediatric patients less than 15 years old. As more diagnostic tests are being developed in the field of virus detection to enhance the detection of infection, its importance in the sero-surveillance activity and assistance in the control and prevention of the infection is indisputable.

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