HOSPITAL-BASED SURVEILLANCE OF JAPANESE ENCEPHALITIS AT A TERTIARY HOSPITAL IN MANILA

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Abstract. Japanese encephalitis virus (JEV) is endemic in the Philippines but the incidence and burden of disease are not well established. We conducted a prospective hospital-based study at San Lazaro Hospital, a tertiary level hospital in Manila, from September 2005 to December 2006. Cases were determined using an in-house dengue and Japanese encephalitis (JE) enzyme-linked immunosorbent assay in order to detect the proportion of JE cases among the acute encephalitis syndrome (AES) cases admitted to our hospital. Fifteen patients were found to have AES, of whom 6 (40%) had confirmed JE. Of the JE cases, 4 were females and 2 were males with an age range of 3-14 years. Three of the 6 JE cases occurred during July. The most common signs and symptoms on admission among JE cases were: fever, headache, loss of appetite, neck rigidity and altered sensorium. JE likely comprises a significant proportion of hospitalized AES cases among children from Manila and nearby provinces. Further studies on the nation-wide prevalence and distribution of JE in the Philippines are needed to guide health authorities in disease control and prevention strategies.

Keywords: Japanese encephalitis, hospital-based study, Philippines

INTRODUCTION

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus infection associated with pig farming and rice cultivation (Campbell *et al*, 2010). *Culex tritaeniorhynchus* mosquitoes, the vector for JE, lay their eggs in rice paddies. Pigs and aquatic birds serve as principal vertebrate amplifying hosts (Halstead *et al*, 2008) with humans generally considered as dead-end hosts.

JE is an important but possibly underestimated cause of morbidity and mortality in Asia; a review by Campbell *et al* (2010) reported the overall incidence of JE was 1.8 per 100,000 population, of which only about 10% of that is reported to the World Health Organization (WHO).

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Although most infections are thought to be asymptomatic (Solomon *et al*, 1998), approximately 1 out of 300 episodes of JE results in encephalitis, with 20-40% mortality (Vaughn and Hoke, 1992). Of those who develop encephalitis and survive, 50% will have neurologic sequelae (Burke *et al*, 1985; Whitley and Gnann, 2002). In Southeast Asia, approximately 30,000-50,000 cases are reported annually with approximately 10,000 deaths (Solomon *et al*, 2000; Tsai, 2000).

JE is endemic in the Philippines. The occurrence of JE was first suggested by Hammon *et al* (1958) and the first isolation of the virus in the Philippines was reported in 1980 (Ksiazek *et al*, 1980; Trosper *et al*, 1980). Continuous transmission of Japanese Encephalitis Virus (JEV) in the Philippines has been shown by human, entomologic, virological and serological studies conducted over the past 50 years (Hammon *et al*, 1958; Venzon *et al*, 1972; Ksiazek *et al*, 1980; Trosper *et al*, 1980; Chan and Samaniego, 1983; Hayes *et al*, 1986; Barzaga, 1989; San Luis *et al*, 1990; Shultz and Hayes, 1993; Natividad *et al*, 2006). Other factors, such as the presence of pig farms and rice fields and the wide distribution of the mosquito vector which are conducive for sustained transmission are present in many parts of the Philippines, especially in the rural areas.

The clinical diagnosis of JEV infection is difficult since it can be subclinical or may present as a non-specific febrile illness, meningo-encephalitis, aseptic meningitis or a polio-like acute flaccid paralysis (Solomon and Winter, 2004; Gould and Solomon, 2008). Laboratory diagnosis of JE is usually based on the presence of antibodies in the blood or CSF of patients (Burke and Nisalak, 1982; Burke *et al*, 1982) with viral isolation mostly performed on the CSF. Definitive laboratory diagnosis of JE is difficult since viremia is usually low and transient and cross reactions may occur due to the presence of antibodies against other co-circulating flaviviruses (Innis *et al*, 1989). A study conducted by Venzon *et al* (1972) using paired serum samples testing for the presence of hemagglutination inhibition antibodies to arbovirus antigens found 17% of 114 cases of encephalitis in the Greater Manila area were caused by flavi (group B) arboviruses, of which JEV is a member of.

In the absence of a national program specific for JE surveillance, accurately estimating the incidence of JEV infection in the Philippines remains a challenge. To help address this scarcity of JEV surveillance information, a prospective hospital-based study was conducted at San Lazaro Hospital (SLH) to determine the proportion of laboratory confirmed JE cases among Acute Encephalitis Syndrome (AES) cases admitted from September 2005 to December 2006.

MATERIALS AND METHODS

Study site

San Lazaro Hospital is a tertiary, government hospital located in Metro Manila. It is the government's national referral center for infectious diseases and treats cases from Metro Manila and other surrounding regions.

Study design and selection of patients

This was a prospective hospital-based surveillance study of patients aged ≥2 years admitted from September 2005 to December 2006 at SLH for AES, defined as a patient admitted to the hospital with acute onset fever (temperature ≥ 38ºC) and a change in mental status (with symptoms such as confusion, disorientation, coma or inability to talk) and/or new onset seizures

except for simple febrile seizures. Patients with explainable causes of AES, AES preceded or associated with exanthem, or patients with known pregnancy were excluded from the study. Patients who met study criteria were informed about the study and requested to participate. Patients were assigned a unique subject ID number and a standardized case report form (CRF) was used to capture demographic information, clinical symptoms, results of imaging studies, travel history, vaccination history, past medical history, clinical laboratory data, daily clinical course, discharge diagnosis and post-discharge clinical condition. This study was approved by the ethics review boards of SLH and the Walter Reed Army Institute of Research (WRAIR).

Data management, specimen collection, storage and transportation

CRF captured admission demographics, clinical, epidemiological, and laboratory data, and subsequent daily clinical progress, discharge diagnosis and a post-discharge clinical condition. These were encoded in a database and manually validated with the CRF and source documents.

Patients satisfying inclusion criteria were requested to provide acute and convalescent blood samples. An acute cerebrospinal fluid (CSF) sample was evaluated if it was obtained for clinical reasons. A convalescent serum sample was drawn 1) ≥5-7 days after the acute serum sample or 2) ≥7 days after onset of illness or 3) ≥3 days after fever defervescence, with the priority of obtaining the convalescent serum sample based on this order. Samples were frozen at -70ºC and shipped on dry ice to the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand and other designated laboratories for further confirmatory testing.

Laboratory testing

Initial testing of acute and convalescent (if available) serum and CSF samples (if available) for JEV was conducted at SLH using an AFRIMS in-house dengue/JE enzyme linked immunosorbent assay (ELISA) (Innis *et al*, 1989). Serum samples with IgM ≥40 units was considered as a likely infection with either dengue or JE. An anti-dengue IgM to anti-Japanese encephalitis IgM ratio ≥1.0 was considered as probably a dengue infection and a ratio <1.0 was considered as probably JEV infection. CSF specimens with an anti-JE IgM ≥40 units was considered as probably JE encephalitis. Serum samples positive for anti-JE IgM were considered as a "recent JE virus infection". Acute serum samples were also tested for dengue via RT-PCR/Nested PCR. This test was performed at AFRIMS using a hemi-nested protocol and primers specific for the C-preM region. The first RT PCR step was combined into one reaction using consensus downstream (anti-sense) and upstream (sense) primers that covered dengue virus (DV) serotypes 1-4. The RT PCR product was then re-amplified in a second step reaction containing 5 primers: the original upstream (sense) primer and 4 internal, serotype specific, anti-sense primers. The nested PCR products were analyzed by gel electrophoresis and the serotype of the DV was identified by the size of the PCR product.

Aliquots of the same sample were sent for confirmation to AFRIMS, Bangkok, Thailand, using the same dengue/JE ELISA. All routine clinical hematology and chemistry studies were done at the SLH clinical laboratory.

Statistical analysis

Measures of location were estimated by mean and median and measures of

Table 1
Signs and symptoms on admission and discharge and laboratory parameters of JE cases (n=6).

Signs and symptoms and laboratory parameters	No. (%)
Signs and symptoms on admission	
Fever	6 (100)
Headache	6 (100)
Loss of appetite	6 (100)
Neck rigidity	5 (83)
Altered sensorium	4 (67)
Hypertonia/spasticity/rigidity	3 (50)
Abdominal pain	3 (50)
Seizures	3 (50)
Motor deficits	2 (33)
Vomiting	2 (33)
Signs and symptoms on discharge	
Not oriented to place, person or time	5 (83)
Needs assistance when standing	4 (67)
Seizures	3 (50)
Abdominal pain	3 (50)
Abnormal gait	2 (33)
Abnormal speech	2 (33)
Vomiting	2 (33)
Cannot sit up without support	1 (17)
Motor deficits	1 (17)
Laboratory parameters	Median (range)
Max peripheral leukocyte count (cells/mm^3)	10.4 (7.7 - 17.5)
Max peripheral neutrophils (%)	80.5 (45 - 87)
Max peripheral lymphocytes (%)	32 (12 - 55)
CSF glucose (mg/dl)	4.3 (2.1 - 6.7) (n=4)
CSF total protein (mg/dl)	0.6 (0.2 - 2) (n=4)
CSF PMN (%)	1 (0 - 12) (n=3)
CSF lymphocytes (%)	93.5 (88 to 99) (n=2)
Max peripheral leukocyte count (cells/mm^3)	10.4 (7.7 - 17.5)
Max peripheral neutrophil (%)	80.5 (45 - 87)

PMN, polymorphonuclear leukocyte.

dispersion were estimated by range using SPSS software version 11.0 (SPSS, Chicago, IL).

RESULTS

In this prospective hospital based study of AES at SLH from September 2005 to December 2006, 15 patients satisfied the inclusion criteria for AES and were enrolled in the study. Eight patients had both acute and convalescent serum samples, 7 had only acute serum samples, 1 had both acute and convalescent CSF samples, 9 had only acute CSF samples obtained and 5 had no CSF sample obtained.

Six (40%) of the 15 AES patients were diagnosed as having JEV encephalitis. Of the 6 JE cases, 4 were females and 2 were males and were aged 3-14 years old with a median age of 8.5 years.

All the JE cases had low to moderate grade fever on admission with a duration of fever ranging from 10 to 20 days. The duration of hospital stay was 11 - 31 days with median stay of 19.5 days. All the JE cases presented in 2006 with 3/6 (50%) being admitted in July and 1 case each being admitted in February, August and November. Maximum temperature during hospitalization had a median of 38.8ºC and range of 38ºC to 39.8ºC. The signs and symptoms on admission and discharge and the CSF results are shown in Table 1. Seizures were reported in 3/6 (50%) of the JEV cases and residual symptoms were present at hospital discharge (Table 1). For the 9 other AES cases diagnosed as non-JEV, 1 was diagnosed as having acute dengue infection via the in-house dengue/JE ELISA, (DEN-3 serotype by PCR); 4 had no evidence of flavivirus infection, 3 had no serological diagnosis (single serum submission), and 1 had neither anti-JE nor anti-dengue IgM detected.

DISCUSSION

Clinically diagnosing JE is challenging since the infection can present as a non-specific febrile illness or present with more severe disease, such as meningoencephalitis, aseptic meningitis or a polio-like acute flaccid paralysis (Solomon *et al*, 1998; Solomon and Vaughn, 2002; Solomon and Winter, 2004; Gould and Solomon, 2008). Five of the 6 cases of JE in our study were from rural areas associated with rice production and the presence of pig farms, similar to other studies (Solomon *et al*, 2000).

In this study, 6 of 15 cases of AES (40%) were diagnosed with JE, suggesting that JE is an important cause of encephalitis at SLH. All of our JE cases were aged less than 15 years, comparable to other studies (Solomon, 2004; Halstead and Jacobson, 2008) where JE attack rates were reported to be higher among children aged 3-15 years. All the JE cases in our study had some form of neurologic sequelae on discharge (Table 1) which highlights the high morbidity caused by JEV infection among children (Kumar *et al*, 1990; Solomon, 2004).

JE transmission in the Philippines has been reported to occur during most months of the year with infrequent, sporadic JE cases and have a broad seasonal peak, which usually occurs during July to September, coinciding with the rainy season (Natividad *et al*, 2006). This peak may be related to irrigation practices (Burke *et al*, 1985; Hayes *et al*, 1986; Vaughn and Hoke, 1992) or to an increase in the number of mosquito vectors (Barzaga, 1989). In our study 3 of 6 JE cases presented during July 2006. However, due to the small sample size, definite conclusions cannot be made about the seasonal variability of JE.

Although JE is endemic in the Philippines, JE vaccines are not locally available and vector control efforts are not commonly practiced. No major outbreaks have been reported in recent years but this may be due to inadequate surveillance. AES data is being collected by the Department of Health but the proportion of JE cases among these cases is difficult to estimate since confirmatory laboratory testing for JEV infection is not routinely performed.

Vaccination is a central component for effective long-term prevention and control efforts. A formalin-inactivated vaccine for JE has been available for more

than 30 years and is effective (Hoke *et al*, 1988) and a live attenuated vaccine requiring fewer doses has been developed and used extensively in China (Hennessy *et al*, 1996; Solomon *et al*, 2000) with good seroconversion and efficacy after 1 dose (Bista *et al*, 2001; Ohrr *et al*, 2005; Tandan *et al*, 2007). Newer generation JE vaccines with excellent safety profiles and effectiveness at lower doses are also available (Halstead and Thomas, 2010a,b). A study conducted in the Philippines showed the live attenuated JE vaccine and measles vaccine administered together was well tolerated and immunogenic in infants less than 1 year old (Gatchalian *et al*, 2008). Despite the commercial availability of safe, effective JE vaccines, they are largely underused in the Philippines.

Although confirmed JE infections have been documented in various locations in the Philippines (Natividad *et al*, 2006), infections may vary substantially by location due to factors associated with JE transmission. Transmission may occurr in provinces considered as "non-endemic" but remain undetected since symptoms might not warrant hospitalization or the case might be misdiagnosed and hence not reported. Data is needed to determine the proportion of JEV infections constituting encephalitis, meningitis and meningoencephalitis cases. JE high risk areas need to be identified. Better and more comprehensive population-based surveillance studies coupled with diagnostic laboratory testing are needed to provide a more accurate picture of the burden of JE in the Philippine population so that adequate vaccine implementation and virus control efforts may be developed.

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