Letter to the Editor

A Centralized Report on Pediatric Japanese Encephalitis Cases from Beijing Children's Hospital, 2013*

LI Jiu Wei[1,^], GAO Xiao Yan[2,3,^], WU Yun[1], FU Shi Hong[2,3], TAN Xiao Juan[4], CAO Yu Xi[2,3], ZHANG Wei Hua[1], YIN Zun Dong[5], HE Ying[2,3], LI Yi Xing[5], LIANG Guo Dong[2,3], XU Wen Bo[4], FANG Fang[1,#], and WANG Huan Yu[2,3,#]

Fifteen pediatric cases of suspected Japanese encephalitis (JE) were reported in Beijing Children's Hospital during the late summer of 2013. The clinical manifestations in most cases included high fever, seizures, and abnormal magnetic resonance imaging findings. Twelve of 15 cases were laboratory-confirmed as JE cases by pathogen identification. Epidemiological investigations showed that five of the 12 laboratory-confirmed patients had an incomplete JE vaccination history. Follow-up investigations after discharge indicated that seven laboratory-confirmed JE patients without JE vaccinations had relatively poor prognoses, with an average Modified Rankin Scale (MRS) score of 2.6 when compared with the other five laboratory-confirmed, JE-vaccinated patients with an average MRS score of 0.5. The observation of pediatric JE cases among those with a history of JE vaccination warrants further attention.

Key words: Japanese encephalitis; Pediatric; Prognoses

Japanese encephalitis (JE), one of the most serious viral encephalitis, is a mosquito-borne encephalitis induced by infection with Japanese encephalitis virus (JEV), which belongs to the genus *Flavivirus*, family Flaviviridae. JE remains a major health problem worldwide, especially in Asia, the Western Pacific, and Northern Australia, with approximately 70,000 cases and 15,000 deaths reported annually; 30%-50% survivors live with irreversible neurological damage[1]. Infection with JEV is often asymptomatic, and children under 15 years of age are principally affected in endemic areas[2].

JE immunization was initially implemented in the eastern coastal areas or relatively economically developed provinces in China since 1968 and has been included in 28 provinces (excluding Qinghai, Xinjiang, and Tibet) in national immunization programs since 2008[2]. At the same time, improvements in laboratory diagnosis of JE have strengthened the quality of the clinical reports of JE cases. In recent years, there has been a marked decrease in the number of JE cases, from 10,308 in 1996 to 2,541 in 2010[3].

According to JE incidence, Hebei Province is a low-endemic area in China with an annual reported number of JE cases ranging from 12 to 37 from 2007 to 2010. However, a JE outbreak of 234 cases was observed in Hebei Province in 2013. A similar situation was also observed in other areas of China, such as Shandong Province, with 407 cases reported in 2013[4]. Owing to the close geographical distance between Hebei and Beijing and the better medical environment in Beijing, most serious cases, such as encephalitis, are referred to Beijing for medical treatment. Herein, we report the results of a systematic study of a cluster of pediatric JE patients

doi: 10.3967/bes2016.121

*This work was supported by grants from the National Key Research and Development Program (2016YFD0500401); a clinical technological innovation project supervised by the Beijing Hospital Authority (XMLX201401); a Development Grant of the State Key Laboratory of Infectious Disease Prevention and Control (2015SKLID505, 2014SKLID03); Ministry of Science and Technology of the People's Republic of China (No. 2013ZX10004202, No. 2013ZX10004101, No. 2012ZX10004215); the National Natural Science Foundation of China (81290342 and 81501757); and the Special National Project on Research and Development of Key Biosafety Technologies (2016YFC1201900).

1. Neurology Department, Beijing Children's Hospital Affiliated to Capital Medical University, Beijing 100045, China; 2. Department of Viral Encephalitis, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 3. State Key Laboratory for Infectious Disease Prevention and Control (SKLID), Chinese Center for Disease Control and Prevention, Beijing 102206, China; 4. Department of Polio, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 5. National Immunization Programme, Chinese Center for Disease Control and Prevention, Beijing 100050, China

reported in Hebei but hospitalized in Beijing Children's Hospital, including their clinical manifestations, clinical outcomes, laboratory test results, JE vaccination history, and prognoses after discharge.

In accordance with the manual for JE diagnosis from the World Health Organization (WHO)[5], we used the following definition in this study. A case of acute encephalitis syndrome (AES) was defined as a person of any age, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures). Suspected JE was defined as an AES case that occurred during the JE epidemic. Laboratory-confirmed JE was defined as a suspected JE case for which there was laboratory confirmation of the presence of JEV-specific immunoglobulin M (IgM) in a single cerebrospinal fluid (CSF) or serum sample, detection of JEV RNA in the CSF using hemi-nested reverse transcriptase polymerase chain reaction (hnRT-PCR), or the isolation and identification of JEV from CSF.

In this study, 15 pediatric patients with suspected JE were hospitalized in Beijing Children's Hospital from September 5 to October 8, 2013. They were all from Hebei Province, neighboring Beijing. The details of the patients' history and clinical findings were recorded on standardized forms. Fever, seizures, behavioral abnormalities, focal weakness, and reflex changes were noted in all patients. The level of consciousness was assessed using the Glasgow coma scale (GCS). The patients showed a broad spectrum of clinical presentations ranging from brief illness lacking specific features to a protracted course of illness with varying severity. The clinical features of the 15 cases are shown in Table 1. High or moderate fever was the most common clinical feature and was observed in all cases. History of seizures was reported in the majority of patients (86.67%, 13/15), and 10 of the 15 children had seizures within 3 days of illness onset. The GCS scores ranged from 3 to 15 (mean, 10.8 ± 1.32) at the time of hospitalization.

In cases of encephalitis, magnetic resonance imaging (MRI) is used to detect and evaluate the extent of lesions and to confirm or exclude a specific diagnosis. In this study, all patients underwent MRI of the brain using a 3.0 T scanner (Magnetom Avanto with Tim system; Siemens, Erlangen, Germany). T1-weighted [repetition time (TR)/echo time (TE)/excitation = 500/50/3 ms], proton density (TR/TE/excitation = 2000-2500/15-20/1 ms), and T2-weighted (TR/TE/excitation = 4000/80-90/1 ms) images were obtained. Susceptibility-weighted and diffusion-weighted imaging were performed in multiple planes. From the results of MRI (Table S1 in the website of BES, www.besjournal.com), the lesions appeared hyperintense in fluid attenuated inversion recovery (FLAIR) and T2-weighted images and isointense to slightly hypointense in T1-weighted images. The MRI results were more frequently abnormal with involvement of the thalamus (12/15) and neocortex (8/15). Involvement of the basal

Table 1. Clinical Manifestations of Fifteen Pediatric Cases with Suspected JE

Patients No.	Sex	Age	Location	Fever	GCS	Seizure Occurred
1	F	6	Langfang	2-4 times/d, T_{max} 40.2 °C	6	From 3th day after onset
2	F	8	Langfang	2 times/d, T_{max} 39.0 °C	8	—
3	F	9	Langfang	2-3 times/d, T_{max} 38.5 °C	6	onset
4	F	4	Langfang	3-4 times/d, T_{max} 40.0 °C	4	From 3th day after onset
5	M	11	Langfang	2-3 times/d, T_{max} 40.5.0 °C	4	From 3th day after onset
6	F	5	Handan	1-3 times/d, T_{max} 39.0 °C	3	From 3th day after onset
7	F	10	Baoding	2-3 times/d, T_{max} 39.0 °C	3	From 5th day after onset
8	M	6	Baoding	2-3 times/d, T_{max} 40.0 °C	6	From first day after onset
9	F	7	Langfang	2-3 times/d, T_{max} 39.0 °C	10	—
10	F	8	Langfang	2-3 times/d, T_{max} 38.5 °C	6	From 3th day after onset
11	M	3	Baoding	3 times/d, T_{max} 39.7 °C	4	From 2nd day after onset
12	F	6	Shijiazhuang	1-3 times/d, T_{max} 40.0 °C	4	From 10th day after onset
13	F	6	Baoding	5-6 times/d, T_{max} 41.0 °C	13	From 4th day after onset
14	M	4	Baoding	2-3 times/d, T_{max} 40.1 °C	7	From 3th day after onset
15	M	2	Baoding	3-4 times/d, T_{max} 39.7 °C	15	From 3th day after onset

Note. F: female; M: male; GCS: the Glasgow coma scale; '—': no seizure.

ganglia and brainstem was observed in four patients. A hippocampal lesion was noted in one patient. MRI changes with thalamic involvement were closely correlated with the presence of dystonia. However, other clinical parameters, such as behavioral abnormalities, seizure, level of coma, and death, were not significantly correlated with radiological abnormalities.

In total, 13 serum and 13 CSF samples were collected during the acute phase from 15 patients after admission. In addition, six serum samples were collected during the convalescing stage. CSF samples were examined for protein, glucose, cells, bacteria, and fungi. The results showed significantly increased mean protein level (492.7 ± 46.9 mg/L) and white blood cell count [(64.4 ± 18.6) × 10^6/L] (Table S1 in the website of BES, www.besjournal.com).

CSF and serum specimens were tested for suspected pathogens, including serological and molecular biological tests, virus isolation, and plaque reduction neutralization tests (PRNT). Based on the clinical diagnosis, JEV was the first pathogen to be tested. All serum samples were tested using a Panbio Japanese Encephalitis/Dengue IgM combo enzyme-linked immunosorbent assay (ELISA) kit (Panbio Diagnostics, Brisbane, Australia) and Beixi Japanese Encephalitis IgM ELISA kit (Shanghai B&C Biological Technology Co., Ltd., Shanghai, China) for JEV-specific antibodies. All CSF samples were tested using a Beixi Japanese Encephalitis IgM ELISA kit. For JEV IgM-negative specimens, we used ELISA IgM kits (Virion/Serion Co., Wurzburg, Germany) for other viral encephalitis pathogens, including echovirus (ECHOV), coxsackievirus (COXV), mumps virus, herpes simplex virus type 1 (HSV-1), HSV-2, varicella zoster virus (VZV), and measles virus. All procedures were performed in accordance with the respective manufacturer's specifications. The results showed that 12 of the 15 patients had serum and/or CSF samples positive for anti-JEV IgM (Table 2). These 12 patients were defined as laboratory-confirmed JE cases. The other three patients negative for anti-JEV IgM were also negative for IgM antibodies to other etiological agents, including HSV, ECHOV, COXV, VZV, mumps virus, and measles virus (data not shown).

In this study, fever was the most common feature and was observed in all laboratory-confirmed JE cases. A history of seizures was observed in 83.3% (10/12) of JE patients, similar to previous studies. Additionally, no obvious difference was observed in the clinical manifestations between JE-positive and unknown cases. Combined with the MRI results, thalamic lesions were observed by MRI in 83.3% (10/12) of laboratory-confirmed JE patients, concordant with previous reports.

Thirteen CSF samples from 15 patients were examined by RT-PCR. RNA was extracted using a QIAamp viral RNA extraction kit (QIAGEN, Valencia, CA) in accordance with the manufacturer's protocol, and first-strand cDNA was produced using Ready-To-Go You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Piscataway, NJ) as described by the manufacturer. The JEV *prM* gene was amplified using hnRT-PCR. The target genes of EV71, CA16, and other enteroviruses were amplified using RT-PCR. One positive and one negative control were included in the reactions, and anticontamination procedures were strictly enforced. All of the procedures were performed according to the manufacturer's specifications. Negative results were obtained for the genomes of suspected pathogens (Table 2), likely owing to the short period of viremia and low virus titer.

Leftover CSF specimens from 10 patients were tested for the presence of viruses as follows. The CSF samples were inoculated into monolayers of BHK-21 and C6/36 cells and incubated at 37 °C and 28 °C, respectively. Cells were observed for a cytopathic effect (CPE) daily from days 1 to 7 after inoculation. Specimens that caused a CPE in three successive cell passages were regarded as positive isolates. No positive results were observed.

In accordance with the WHO recommendations, JE can be confirmed if a four-fold or greater increase in neutralizing anti-JEV antibody is observed in serum collected during the convalescent phase compared with those during the acute phase by PRNT. In this study, PRNT was performed on six convalescent sera and three available acute sera. Sera were tested in serial two-fold dilutions from 1 to 10. Diluted sera were mixed with equal volumes of culture medium containing JEV [P3 strain, 100 plaque-forming units (PFU)] and incubated at 37 °C for 1 hour. Six-well plates of confluent BHK-21 cells were inoculated with the serum-virus mixtures and incubated at 37 °C in a 5% CO_2 incubator for 1 h. Plates were overlaid with 3 mL of medium containing 0.8% agarose and again with 2.5 mL of second overlay medium containing neutral red vital stain (Sigma-Aldrich, St. Louis, MO). The neutralizing antibody titer was identified as the highest serum dilution that reduced the number of virus plaques in

Table 2. Pathogen Identification of Fifteen Pediatric Cases with Suspected JE in Beijing Children's Hospital

No	Specimen Type	Onset Date	Specimen Collection Date		ELISA		rRT-PCR			PRNT		Virus Isolation	Final Identification
			S1	S2	Beixi JE IgM	Panbio JE/DEN Combo	EV71/CA16	Other EV	JEV	S1	S2		
1	Serum	2013/9/6	2013/9/11	2013/9/26	POS	POS	—	—	—	1:40	1:80	—	JE
	CSF		2013/9/11		POS		NEG	NEG	NEG	—	—	NEG	
2	Serum	2013/9/14	2013/9/26		POS	POS	—	—	—	—	—	—	JE
	CSF		2013/9/22		POS		NEG	NEG	NEG	—	—	—	
3	Serum	2013/9/16	2013/9/25		NEG	NEG	—	—	—	—	—	—	Unknown
	CSF		2013/9/25		NEG		NEG	NEG	NEG	—	—	—	
4	Serum	2013/9/16	2013/9/22	2013/10/18	POS	POS	—	—	—	1:80	1:320	—	JE
	CSF		2013/10/18		POS		NEG	NEG	NEG	—	—	NEG	
5	CSF	2013/9/16	2013/9/20		NEG		NEG	NEG	NEG	—	—	NEG	Unknown
6	Serum	2013/9/20	2013/9/25		POS	POS	—	—	—	—	—	—	JE
7	Serum	2013/9/21	2013/9/25		POS	POS	—	—	—	—	—	—	JE
	CSF		2013/9/25		POS		NEG	NEG	NEG	—	—	—	
8	Serum	2013/9/17	2013/9/22		POS	POS	—	—	—	—	—	—	JE
9	Serum	2013/9/19	2013/9/25		POS	POS	—	—	—	—	—	—	JE
	CSF		2013/9/25		POS		NEG	NEG	NEG	—	—	NEG	
10	Serum	2013/9/29	2013/10/6	2013/11/1	NEG	NEG	—	—	—	1:20	1:40	—	Unknown
	CSF		2013/10/6		NEG		NEG	NEG	NEG	—	—	NEG	
11	CSF	2013/9/23	2013/9/29		POS		NEG	NEG	NEG	—	—	NEG	JE
12	Serum	2013/9/5	2013/10/2	2013/11/1	POS	POS	—	—	—	—	1:160	—	JE
	CSF		2013/10/2		POS		NEG	NEG	NEG	—	—	NEG	
13	Serum	2013/9/24	2013/9/30		POS	POS	—	—	—	—	—	—	JE
	CSF		2013/9/30		POS		NEG	NEG	NEG	—	—	NEG	
14	Serum	2013/10/8	2013/10/12	2011/11/1	POS	POS	—	—	—	—	1:160	—	JE
	CSF		2013/10/12		POS		NEG	NEG	NEG	—	—	NEG	
15	Serum	2013/9/28	2013/9/30	2013/11/1	POS	POS	—	—	—		1:40	—	JE
	CSF		2013/9/30		POS		NEG	NEG	NEG	—	—	NEG	

Note. '—' means undone; rRT-PCR was conducted on 13 patients with available CSF and virus isolation was carried out on 10 patients with leftover CSF; PRNT was conducted on three paired sera and three sera collected on convalescent phage; POS, positive; NEG, negtive; JE, Japanese encephalitis; S1, acute stage; S2, convalescing stage.

the test by ≥ 90%. The results showed a four-fold increase in only one confirmed anti-JE IgM antibody-positive case (No. 4). No such increase was observed in the other two cases for which both convalescent and acute sera were available (Nos. 1 and 10). The neutralizing antibody titers of the other three cases for which only convalescent sera were available were 1:160, 1:160, and 1:40, respectively. The lack of a four-fold increase between convalescent and acute sera may have been owing to delayed specimen collection, as most of these cases were from rural areas and experienced complex hospitalization processes before their admission to Beijing Children's Hospital.

Epidemiological retrospective investigations including health conditions, JE vaccination history, and prognosis after discharge were conducted. Information on outcome was recorded at 1, 3, 5, and 12 months after discharge from the hospital. The Modified Rankin Scale (MRS) was used to evaluate neurological function and outcome: 0 = no symptoms, 1 = no significant disability (able to carry out all usual activities despite some symptoms), 2 = slight disability (able to look after their own affairs without assistance, but unable to carry out all previous activities), 3 = moderate disability (requires some help, but able to walk independently), 4 = moderately severe disability (unable to attend to own bodily needs without assistance, and unable to walk independently), 5 = severe disability (requires constant nursing care and attention, bedridden, incontinent), and 6 = dead.

Among the 15 pediatric patients, 14 were healthy before onset; one laboratory-confirmed JE patient had undergone resection of a cerebellar medulloblastoma 2 years previously and had recovered before the JE infection (No. 11) (Table 1).

Three patients died during the acute phase of illness, and all of the others patients were alive at the time of writing this report. Thus, the mortality rate was 20% (3/15). Moreover, two deaths among 12 patients with laboratory-confirmed JE were observed, representing a mortality rate of 16.67% (2/12), similar to previous reports. For example, the JE outbreak in Yuncheng City, Shanxi province had a mortality rate of 18%[6], and a 6-month retrospective survey of patients with JE in Shandong Province, China reported a mortality rate of 17.7%-19.3%[7]. However, the annual mortality rate reported in the notifiable disease system ranged from 2.51% to 4.66%[8]. The lower reported mortality rate compared to the actual mortality rate is probably owing to a lack of follow-up and the occurrence of death after discharge. One laboratory- confirmed JE patient (No. 1) developed secondary anti-*N*-methyl-D-aspartate-receptor (NMDA-R) encephalitis 2 months after the onset of JE.

Six of the patients had a definite JE vaccination history (Table 3). Among them, five were laboratory-confirmed cases of JE. Of the five laboratory-confirmed JE patients with JE vaccination history, four had a single dose of the vaccine only and one had had two doses. This finding indicated that incomplete JE vaccination or only one dose may result in vaccination failure. The three patients who died had no history of JE vaccination.

The follow-up investigation (Table 3) showed that in the first month, the average MRS score of all patients was 3.7. At the 3-month follow-up, all of the patients showed an average MRS score reduction to 2.9. At the 5-month follow-up, the average MRS score of all patients was 2.3, and four had made a full recovery. At the 12-month follow-up, the average MRS score was 2.1, and six patients had fully recovered; five of these six fully recovered patients were laboratory-confirmed cases of JE. Moreover, among these five laboratory-confirmed patients, three had received JE vaccination several years previously; the MRS scores of the other two JE patients were 1 and 2, respectively. This finding indicated that three of the five laboratory-confirmed JE patients who had been vaccinated for JEV made full recoveries, and while the other two had better outcomes, with MRS scores of 1 and 2 at the 12-month follow-up, respectively. Although the outcomes did not differ significantly, the prognosis was relatively poor in the other seven laboratory-confirmed JE patients with no JE vaccination history, including two deaths, one with an MRS score of 5, two with an MRS score of 1, and two full recoveries at the 12-month follow-up.

Hebei Province administers a live attenuated SA14-14-2 JE vaccine with an immunization schedule of two doses, at 8 months and 2 years old, respectively[9]. In this study, a few patients with JE had a JE vaccination history, suggesting that vaccination failure may have occurred owing to incomplete vaccination. However, the prognosis of patients with JE with a vaccination history was better than that of patients without vaccination, showing that vaccination could influence the clinical features and protect the patient from a severe clinical presentation even with only one dose. Most of the patients had no JE vaccination history, indicating

Table 3. JE Vaccination History and Prognosis Investigation of Fifteen Pediatric Cases with Suspected JE in Beijing Children's Hospital

Patients No.	Age	Final Identification	PRNT Titer		JE Vaccination History			Prognosis (MRS)			
			S1	S2	Vaccine Type	Doses	Last Vaccination Date	1th month	3th month	5th month	12th month
1	6	JE	1:40	1:80	/	/	/	5	5	5	5
2	8	JE	—	—	/	/	/	1	0	0	0
3	9	Unknown	—	—	/	/	/	5	4	4	3
4	4	JE	1:80	1:320	/	/	/	5	2	1	1
5	11	Unknown	—	—	/	/	/	6	6	6	6
6	5	JE	—	—	/	/	/	6	6	6	6
7	10	JE	—	—	/	/	/	6	6	6	6
8	6	JE	—	—	SA14-14-2	2	2008/04/28	5	4	2	1
9	7	JE	—	—	SA14-14-2	1	2007/06/05	2	1	0	0
10	8	Unknown	1:20	1:40	SA14-14-2	1	2005/12/25	2	1	1	0
11	3	JE	—	—	/	/	/	3	2	1	1
12	6	JE	—	1:160	SA14-14-2	1	2009/10/06	3	2	2	2
13	6	JE	—	—	SA14-14-2	1	2008/04/04	2	1	0	0
14	4	JE	—	1:160	SA14-14-2	1	2010/11/14	2	1	0	0
15	2	JE	—	1:40	/	/	/	3	1	1	0

Note. '—' means undone; '/' means no JE vaccination; MRS: Modified Rankin Scale; S1, acute stage; S2, convalescing stage.

that there is still a gap in JEV vaccination coverage. A previous study showed that integrating the JE vaccine into the Expanded Programme on Immunization (EPI) is a cost-effective investment[10]. Therefore, complete vaccination coverage will be in urgent demand.

Although the clinical manifestations of JE cases in this study were similar to those of previous reports, the 12 months follow-up survey after discharge provided valuable data on JE prognoses, especially for the JE cases with a JE vaccination history. However, owing to the limited number of patients, a comprehensive study is necessary to validate these conclusions.

^These authors equally contributed to this manuscript.

#Correspondence should be addressed to: WANG Huan Yu, MD, Tel & Fax: 86-10-58900839, E-mail: rainoffall@yahoo.com; FANG Fang, MD, Tel & Fax: 86-10-59616356, E-mail: 13910150389@163.com

Biographical notes of the first authors: LI Jiu Wei, male, born in 1975, PhD, Associate Professor, majoring in pediatric neurology diseases; GAO Xiao Yan, female, born in 1982, PhD, Associate Professor, majoring in Japanese encephalitis and other arboviruses.

Received: May 22, 2016;

Accepted: November 29, 2016

REFERENCES

1. Campbell GL, Hills SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ, 2011; 89, 766-74.
2. Centers for Disease Control and Prevention. Japanese encephalitis surveillance and immunization -Asia and the Western Pacific, 2012. MMWR Morb Mortal Wkly Rep, 2013; 62, 658-62.
3. Gao XY, Li XL, Li MH, et al. Vaccine Strategies for the Control and Prevention of Japanese Encephalitis in Mainland China, 1951– 2011. PLoS Negl Trop Dis, 2014; 8, e3015.
4. Tao ZX, Liu GF, Wang M, et al. Molecular Epidemiology of Japanese Encephalitis Virus in Mosquitoes during an Outbreak in China, 2013. Sci Rep, 2014; 4, 4908.
5. Manual for the Laboratory Diagnosis of Japanese Encephalitis Virus Infection. Available: http://www.who.int/immunization/monitoring_surveillance/burden/laboratory/JE/en/[2016-5-5].
6. Wang LH, Fu SH, Wang HY, et al. Japanese encephalitis outbreak, Yuncheng, China, 2006. Emerg Infect Dis, 2007; 13, 1123-5.
7. Liu GF, Liu F, Song LZ, et al. Followed-up Investigation for the Clinical Consequence of Japanese Encephalitis Cases. Chin J Vac Immun, 2011; 17, 45-8.
8. Wang HY, Li YX, Liang XF, et al. Japanese encephalitis in mainland China. Jpn J Infect Dis, 2009; 62, 331-6.
9. Halstead S, Jacobson J. Japanese encephalitis vaccines. IN: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 5th edition. Philadelphia: Elsevier, 2008; 311-52.
10. Yin ZD, Beeler Asay GR, Zhang L, et al. An economic evaluation of the use of Japanese encephalitis vaccine in the expanded program of immunization of Guizhou province, China. Vaccine, 2012; 30, 5569-77.

Table S1. Clinical Examination (Head MRI and CSF) of 15 Pediatric Cases with Suspected JE in Beijing Children's Hospital

Patients No.	CSF Testing		Brain MRI
	Protein	WBC	
1	483 mg/L	10×10^6/L	T1-weighted images exhibited hypointensity in both thalamus, brainstem, right temporal lobe and frontal lobe,which appeared to be hyperintense on T2-weighted images.
2	633 mg/L	70×10^6/L	T1-weighted images exhibited hypointensity in both thalamus,which were hyperintense on T2-weighted images.
3	333 mg/L	8×10^6/L	T2-weighted MR images demonstrated slightly hyperintensity in both cerebral hemisphere cortex, putamen, caudate nucleus and inner sides of thalamus, which appeared to be hyperintense on diffuse-weighted images.
4	782 mg/L	120×10^6/L	T1-weighted images exhibited subtle hypointensity in both basal ganglia and thalamus, which were slightly hyperintense on T2-weighted images.
5	451 mg/L	90×10^6/L	T1-weighted images revealed hypointensity in both thalamus and cerebral peduncle and temporal lobe,which were hyperintense on T2-weighted images.
6	-	-	T1-weighted images showed hypointensity in bothl thalamus and the cerebral hemisphere frontoparietal lobe,which were hyperintense on T2-weighted images.
7	728 mg/L	80×10^6/L	T1-weighted images exhibited subtle hypointensity in both thalamus, which were slightly hyperintense on T2-weighted and diffuse-weighted images.
8	803 mg/L	70×10^6/L	T2-weighted MR images demonstrated slightly hyperintensity in both thalamus and left temporal lobe.
9	260 mg/L	110×10^6/L	T2-weighted MR images showed hyperintensity in both cerebral peduncle, thalamus, caudate nucleus and lentiform nucleus.
10	423mg/	7×10^6/L	Findings are normal.
11	1035 mg/L	10×10^6/L	T2-weighted images revealed hyperintensity in both thalamus and parietal lobes, which were slightly hypointense on T1-weighted images.
12	280 mg/L	40×10^6/L	T1-weighted images exhibited isoerintensity in both thalamus, caudate nucleus,midbrain and cerebral peduncle,which were slightly hyperintense on T2-weighted and FLAIR images.
13	374 mg/L	70×10^6/L	T2-weighted images exhibited subtle hyperintensity in both thalamus and right frontal parietal lobes, which were slightly hyperintense on FLAIR images as well.
14	275 mg/L	28×10^6/L	T2-weighted and FLAIR images exhibited hyperintensity in both thalamus and frontal parietal lobes, which were hyperintense on duffse-weighted images,too.
15	535 mg/L	70×10^6/L	Findings are normal.