Japanese Encephalitis Disease Burden and Clinical Features of Japanese Encephalitis in Four Cities in the People's Republic of China

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Abstract. The incidence rate of Japanese encephalitis (JE) in the People's Republic of China has decreased substantially with the wide use of JE vaccine, but the accuracy of JE reporting is uncertain. We established active surveillance for acute meningitis and encephalitis syndrome (AMES) in four prefectures in China during 2006–2008 and performed JE laboratory testing on AMES cases identified from six sentinel hospitals in each prefecture. We estimated JE incidence for each prefecture by applying age-adjusted and season-adjusted JE positivity rates from sentinel hospitals to the total AMES resident cases. We identified 4,513 AMES cases, including 3,561 (79%) among residents of four prefectures. Among 2,294 AMES cases from sentinel hospitals, we identified 213 (9.2%) laboratory-confirmed JE cases. Adjusted estimates of JE incidence per 100,000 persons ranged from 0.08 in Shijiazhuang to 1.58 in Guigang. Active surveillance and laboratory confirmation provides a better estimate of the actual JE disease burden and should be used to further refine JE prevention strategies.

INTRODUCTION

Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, is the most common cause of encephalitis in Asia.^{1,2} Of an estimated 50,000 cases yearly, 5–35% of patients die and 30–50% of survivors have significant neurologic sequelae.^{3,4} The clinical manifestations of JE vary widely from a nonspecific febrile illness to severe encephalitis. It is difficult to distinguish JE from other causes of viral encephalitis without using specific laboratory testing.

Japanese encephalitis is a highly endemic disease in China. Devastating epidemics occurred in the 1960s and 1970s, with the highest reported incidence reaching 20.9 cases/100,000 population in 1970. The incidence rate has decreased substantially since the 1980s, when JE vaccines were introduced for children ≤ 10 years of age.⁴ From 1981 through 2007, JE vaccine was not included in the national Expanded Program on Immunization (EPI), but was made available at provincial and local Chinese Center for Disease Control (CDC) clinics for parents willing

to pay. Most provinces, including Hubei, Hebei and Guangxi, performed annual mass vaccination campaigns before the JE epidemic season. Certain provinces, including Shandong beginning in 1986, included JE vaccines in the routine immunization of children 1–10 years of age. Beginning in 2008, JE vaccine has been included in the national EPI in all disease-endemic areas (including all study sites), with no charge to parents.

Japanese encephalitis is among the 38 routinely reportable infectious diseases in China. The reported national incidence rate has been < 1/100,000 since 1996. A total of 5,000–10,000 JE cases and several hundred deaths are reported annually; > 85% of reported cases occur in children < 15 years of age.³ However, reported JE incidence ranges from < 1/million to 3.5/100,000 in individual provinces,^{5,6} and local outbreaks occur frequently, including among older adults.^{3,7} Because most JE cases are diagnosed clinically and not confirmed by laboratory testing, the accuracy of these surveillance data is uncertain. A better understanding of the incidence and epidemiology of JE in China is important to monitor and refine vaccination programs.

In 2006, the China Ministry of Health, China CDC, World Health Organization, and U.S. Centers for Disease Control and Prevention initiated active surveillance for acute meningitis and encephalitis syndrome (AMES) in one prefecture in each of four provinces. The objectives of the project were to determine the incidence and epidemiology of vaccine-preventable causes of meningitis and encephalitis (i.e., JEV, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*) and to improve the laboratory capacity for diagnosis of these diseases. We report the epidemiology, clinical features, and estimated incidence of JE identified in the four Chinese prefectures during 2006–2008.

MATERIALS AND METHODS

Disease surveillance. The project was started in September 2006 in Jinan Prefecture, the capital of Shandong Province,

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and Yichang Prefecture in western Hubei Province, which have populations of 6.1 and 3.9 million, respectively (Figure 1). In April 2007, the project areas were expanded to Shijiazhuang Prefecture, the capital of Hebei Province, and Guigang Prefecture (population = 4.1 million), in southeastern Guangxi Province. Because of large prefecture size, we selected only 13 counties (population = 5.1 million) as surveillance sites in Shijiazhuang Prefecture. Six hospitals were selected as sentinel hospitals in each prefecture, including the largest general hospital, infectious disease hospital, children's hospital, and one local county hospital. Efforts were made to collect diagnostic specimens (serum and cerebrospinal fluid [CSF]) for laboratory testing from all clinical cases of meningitis and encephalitis syndromes. All other hospitals in each prefecture were designated as non-sentinel hospitals and only limited epidemiologic data were obtained on the reported AMES cases; no specimens were collected or tested under this project.

An AMES case was defined as a person of any age at any time of year with the acute onset of fever with change in mental status and/or meningeal signs (e.g., neck stiffness, headache). The clinic doctors in sentinel and non-sentinel hospitals reported the AMES cases to the county CDC. Public health doctors performed case investigations within 48 hours after receiving case reports from sentinel hospitals. Every month, public health doctors obtained the AMES case information, including name, date of birth, date of onset, and initial clinical diagnosis, which was recorded in the non-sentinel hospitals by the clinic doctors.

Active surveillance was performed every 10 days in sentinel and non-sentinel hospitals. Public health doctors reviewed admission and discharge records in the pediatric, neurology, and infectious disease inpatient and outpatient departments and queried clinic doctors to identify and report any missed AMES cases. Hospital computer files were also reviewed to identify all cases with 12 specific diagnostic codes (initial diagnoses in sentinel hospitals included Japanese encephalitis, viral encephalitis, viral meningitis, viral meningoencephalitis, other

encephalitis, meningococcal meningitis, purulent meningitis, cerebrospinal meningitis, tuberculosis meningitis, tuberculosis meningoencephalitis, other meningitis, and other diagnosis).

Specimen collection and laboratory testing. Doctors in sentinel hospitals obtained blood (≥ 3 mL) and/or CSF samples from the AMES cases. Blood samples were centrifuged and serum separated for further testing. Diagnostic testing at the hospital laboratory (e.g., CSF examination and bacterial culture) was completed according to the usual physicians' requests. All samples (serum or CSF) were stored at $\leq -20^{\circ}\text{C}$. If the initial blood specimen was obtained within 7 days of onset of illness, doctors were asked to collect a second serum specimen for JE IgM testing ≥ 7 days after onset of illness and before discharge from the hospital. Specimens were transported to prefecture CDCs within 48 hours after samples were received.

Prefecture CDC laboratories performed a JEV IgM enzyme-linked immunosorbent assay (ELISA) on all CSF and serum samples by using a JEV IgM capture ELISA kit (Beixa Kit; Shanghai B&C Biological Technology Co., Ltd., Shanghai, China). A subset of specimens was forwarded to provincial and national CDC JE laboratories for quality control testing with the same kit. A laboratory-confirmed JE case was defined as any case that gave a positive result for the IgM ELISA for at least one CSF or serum specimen.

Ethical considerations. Serum and CSF specimens of patients were obtained by sentinel hospitals as part of routine diagnostic testing. Testing performed at reference laboratories was conducted for de-identified specimens. The CSF specimens were obtained in hospitals after signed informed consent was obtained from the patient. The ethical review committee at the China CDC determined that additional informed consent was not required because this study was for surveillance and used standard clinical specimens.

Data collection and analysis. Demographic, clinical, and laboratory data from the sentinel hospitals were entered in an EPIDATA database (<http://www.epidata.dk/history.htm>). Data from non-sentinel hospitals were compiled using standardized

FIGURE 1. Acute meningitis and encephalitis syndrome (AMES) surveillance project areas, People's Republic of China.

Microsoft (Redmond, WA) Excel spreadsheets. We compared the epidemiologic and clinical characteristics of AMES cases and laboratory confirmed JE cases in the four prefectures, and calculated average annual incidence rates based on the different surveillance intervals in the 4 prefectures. To estimate the JE incidence rate in the four prefectures, JE incidence was adjusted on the basis of the positive proportion (according to the age and seasonality) of residents tested for JE in sentinel AMES hospitals and applied to the untested cases in sentinel hospitals and AMES cases in non-sentinel hospitals by the direct method. For comparison of clinical features of JEV IgM-positive and -negative AMES cases and JE cases initially diagnosed as JE compared with those given another initial diagnosis, the chi-square test or Fisher exact test were used for analysis of rates or percentages in different groups and the Student's *t*-test or Kruskal-Wallis test were used for analysis of means.

RESULTS

Overall surveillance by province. From the start of the project through September 2008, a total of 4,514 AMES cases were reported, 2,815 from sentinel hospitals and 1,699 from non-sentinel hospitals (Table 1). The surveillance prefectures in Shandong and Hebei provinces were in provincial capital cities, which had large referral hospitals that received patients from surrounding prefectures. In these prefectures, > 50% of AMES cases identified in sentinel hospitals came from other cities/prefectures (non-residents), whereas most cases identified in non-sentinel hospitals resided in the surveillance areas of the prefectures of Shandong and Hebei. In contrast, the surveillance prefectures in Hubei and Guangxi provinces were in smaller cities, distant from provincial capitals, for which > 90% of AMES cases at sentinel and non-sentinel hospitals were prefecture residents. Overall incidence rates of AMES cases in prefecture residents were similar, approximately 10 cases/100,000 residents, ranging from 9.2/100,000 to 14.2/100,000 (Table 1).

Demographic features of AMES cases and testing for JE. Among AMES cases identified in sentinel hospitals, most (45–77%) were children < 15 years of age, but age distribution differed between the four prefectures. Guigang and Shijiazhuang prefectures had larger proportions of cases among young children compared with the other prefectures

(Table 2). In non-sentinel hospitals in each prefecture, a higher proportion of cases were < 15 years of age (70–85%), and the highest proportions in children 5–14 years of age (53–61%).

Rates of specimen collection for JE testing varied by prefecture from 26% in Shijiazhuang Prefecture to 90% in Guigang Prefecture for the first blood specimen and from 39% (Shijiazhuang) to 72% (Guigang) for CSF. Less than 2% of the cases had a second serum specimen obtained. Overall, either serum or CSF specimens were available for 82% of the cases and varied from 60% to 97% in the four prefectures (Table 2).

Results of JEV IgM ELISA. Overall, 213 (9.3%) AMES cases had laboratory evidence of recent JE infection, ranging from 10 to 93 JE cases in each of the four prefectures (Table 3). The JE positivity rates for serum and CSF samples were 9.7% (177 of 1,825) and 8.3% (138 of 1,661), respectively. Among the tested AMES cases in sentinel hospitals, the lowest rates of positive JE test results were in Shijiazhuang (3.2%) and Yichang (4.2%) and the highest rates were in Guigang (12%) and Jinan (13%). The proportion of AMES cases attributed to JE in each age group also varied by prefecture. In Guigang, 91 (15%) of 620 AMES cases among children < 15 years of age were caused by JE, and 95% of the JE cases were in this age group. A similar age pattern, but less striking, was seen in Yichang. In contrast, in Jinan and Shijiazhuang, JE positivity rates were more uniform in all age groups. Substantial proportions (30–51%) of cases occurred among persons ≥ 15 years of age, including older adults ≥ 30 years of age in Jinan, Shijiazhuang, and Yichang.

Among the tested resident AMES cases, there were 31 JE cases in Jinan, 18 JE cases in Yichang, 1 JE case in Shijiazhuang, and 89 JE cases in Guigang. The JE positivity rates were higher among non-resident cases referred from outside the prefecture than in prefecture residents in Jinan, Shijiazhuang and Guigang.

The seasonality of JE cases differed moderately between the four prefectures. The JE epidemic seasons in Jinan and Shijiazhuang prefectures, which are in northern China, were during August–October. In Yichang prefecture, which is in central/southern China, the JE epidemic season was during July–August. In Guigang Prefecture, which is in southern China, the JE epidemic season was the earliest (June–July) (Figure 2). In each prefecture, only a few cases were identified outside the JE epidemic season.

TABLE 1
Surveillance of acute meningitis and encephalitis cases in four prefectures, People's Republic of China*

Characteristic	Province/prefecture				Total
	Shandong/Jinan	Hubei/Yichang	Hebei/Shijiazhuang†	Guangxi/Guigang	
Population	6,107,497	3,911,518	5,062,679	4,113,366	
Project duration, months	25	25	17	17	
No. AMES cases	1,754	807	1,109	844	4,514
No. cases from sentinel hospitals	830	631	517	837	2,815
No. (%) residents	410 (49.4)	573 (90.8)	228 (44.1)	820 (98.0)	2,031 (72.1)
No. (%) non-residents	420 (50.6)	58 (9.2)	289 (55.9)	17 (2.0)	784 (27.9)
No. cases from non-sentinel hospitals	924	176	592	7	1,699
No. (%) residents	803 (86.9)	176 (100.0)	544 (91.9)	7 (100.0)	1,530 (90)
No. (%) non-residents	121 (13.1)	0 (0)	48 (8.1)	0 (0)	169 (10)
AMES rate in residents/100,000/year	9.53	9.19	10.76	14.19	

* AMES = acute meningitis and encephalitis syndrome.

† Only 13 counties in Shijiazhuang Prefecture were included in the project.

TABLE 2
Demographic features of the AMES cases and specimen collection details in sentinel hospitals, People's Republic of China*

Characteristic	Province/prefecture				Total (n = 2,815), no. (%)
	Shandong/Jinan (n = 830), no. (%)	Hubei/Yichang (n = 631), no. (%)	Hebei/Shijiazhuang (n = 517), no. (%)	Guangxi/Guigang (n = 837), no. (%)	
Age, years					
< 2	98 (11.8)	55 (8.7)	128 (24.8)	239 (28.6)	520 (18.5)
2–4	158 (19.0)	58 (9.2)	70 (13.5)	185 (22.1)	471 (16.7)
5–14	250 (30.1)	170 (26.9)	190 (36.8)	217 (25.9)	827 (29.4)
15–29	137 (16.5)	107 (17.0)	59 (11.4)	56 (6.7)	359 (12.8)
30–44	103 (12.4)	109 (17.3)	26 (5.0)	35 (4.2)	273 (9.7)
> 45	84 (10.1)	132 (20.9)	44 (8.5)	105 (12.5)	365 (13.0)
Sex					
M	508 (61.2)	383 (60.7)	328 (63.4)	514 (61.4)	1,733 (61.6)
F	322 (38.8)	248 (39.3)	189 (36.6)	323 (38.6)	1,082 (38.4)
JE testing					
Blood (first)	590 (71.1)	352 (55.8)	134 (25.9)	749 (89.5)	1,825 (64.8)
CSF	512 (61.7)	341 (54.0)	203 (39.3)	605 (72.3)	1,661 (59.0)
Either	719 (86.6)	457 (72.4)	309 (59.8)	809 (96.7)	2,294 (81.5)

* AMES = acute meningitis and encephalitis syndrome; JE = Japanese encephalitis; CSF = cerebrospinal fluid; Either = either blood or CSF.

Among 213 laboratory-confirmed JE cases, the proportion of cases living in rural areas ranged from 63% in Shijiazhuang to 99% in Guigang. Based on confirmed JE cases in residents in the four prefectures, the crude incidence rates varied from 0.01/100,000 persons in Shijiazhuang to 1.5/100,000 persons in Guigang. After adjustment for JE positivity rates by age and month for tested cases in sentinel hospitals, and by age and month of onset of untested cases in residents, the estimated JE incidence rates in the four prefectures varied more than 10-fold, ranging from 0.08 (Shijiazhuang) to 1.58 (Guigang)/100,000 persons (Table 3).

Among the 2,815 AMES cases identified at sentinel hospitals, 1,194 (42%) were initially diagnosed as viral encephalitis or meningitis and another 645 (23%) were classified as other encephalitis. Based on clinical manifestations alone, JE was the initial diagnosis in 117 (4%) of AMES cases, of which

63 (54%) were confirmed by serologic testing. However, the remaining 150 (70%) of the 213 laboratory-confirmed JE cases were initially diagnosed as other clinical syndromes or etiologies (Table 4). Among the 1,487 AMES cases initially classified as non-JE viral encephalitis, viral meningitis, or other encephalitis, 114 (8%) had laboratory evidence of JE. In contrast, only 8 (3%) of the 272 AMES cases that were initially classified as bacterial or mycobacterial cases based on their clinical manifestations were ultimately diagnosed as JE.

Overall, 631 (34%) of AMES cases had a specimen obtained ≥ 7 days after onset of illness but another 615 (33%) cases had all specimens collected within 2 days of illness onset. Among JE clinically diagnosed and laboratory confirmed cases, 52 (88%) of 59 were from specimens that had been obtained ≥ 3 days after onset. In contrast, 20 (41%) of the 49 cases with

TABLE 3
JEV IgM testing results and positivity rate by age group and province, People's Republic of China*

Characteristic	Province/prefecture			
	Shandong/Jinan (n = 719) % positive (no. positive/no. tested)	Hubei/Yichang (n = 457) % positive (no. positive/no. tested)	Hebei/Shijiazhuang (n = 309) % positive (no. positive/no. tested)	Guangxi/Guigang (n = 809) % positive (no. positive/no. tested)
Total	12.7 (91/719)	4.2 (19/457)	3.2 (10/309)	11.5 (93/809)
Age, years				
< 2	4.7 (4/86)	11.1 (4/36)	1.1 (1/95)	8.3 (19/229)
2–4	12.1 (18/149)	9.3 (4/43)	2.2 (1/46)	30.2 (54/179)
5–14	9.9 (23/233)	3.6 (5/138)	3.8 (5/130)	8.5 (18/212)
15–29	13.0 (13/100)	1.4 (1/70)	4.5 (1/22)	3.8 (2/53)
30–44	18.5 (15/81)	4.2 (3/71)	20.0 (1/5)	0 (0/32)
> 45	25.7 (18/70)	2.0 (2/99)	9.1 (1/11)	0 (0/104)
Sex				
M	11.2 (50/446)	3.8 (11/292)	3.8 (8/210)	10.4 (52/498)
F	15.0 (41/273)	4.8 (8/165)	2.0 (2/99)	13.2 (41/311)
Place of residence				
Residents	8.3 (31/372)	4.4 (18/413)	0.6 (1/154)	11.2 (89/792)
Non-residents	17.3 (60/373)	2.3 (1/44)	5.8 (9/155)	23.5 (4/17)
JE Incidence in residents/year/100,000				
Crude	0.24	0.22	0.01	1.53
Adjusted†	0.71	0.48	0.08	1.58

* JEV = Japanese encephalitis virus.

† Adjusted rate is calculated based on JE positivity among resident cases by age and seasonality applied to all non-tested cases assuming that the subset tested is a representative sample of acute meningitis and encephalitis syndrome cases and the homogenous rate of infection within a given prefecture (age group, time period stratum).

FIGURE 2. Seasonality of Japanese encephalitis cases in the four prefectures, People's Republic of China.

initial clinical diagnosis of JE but with negative JE laboratory testing results, were collected within 2 days of illness onset.

Comparison of clinical features among laboratory-confirmed JE cases and JEV IgM-negative AMES cases. Clinical symptoms and signs and CSF characteristics were compared between the JEV IgM-positive ($n = 213$) and JE IgM-negative AMES cases in a subset of samples in which serum was obtained ≥ 7 days after onset of illness ($n = 553$). Clinical symptoms of laboratory-confirmed JE cases were consistent with classical descriptions, with high proportions with fever and CNS manifestations, including lethargy, drowsiness, consciousness disorders, and convulsions. The proportion of cases with any fever and higher degree of fever was higher in JEV IgM-positive cases than in the JEV IgM-negative AMES cases ($P < 0.01$). In addition, the proportions of patients with lethargy, drowsiness, consciousness disorders, and convulsions, and clinical signs (stiff neck, opisthotonos, and meningeal irritation) were significantly higher in the JEV-IgM positive group than in the JEV IgM-negative encephalitis group ($P < 0.05$). For the CSF features, laboratory-confirmed JE cases had lower leukocyte counts (mean $\pm$ SD = 69.1 ± 98.3 cells/mm³) and higher glucose levels (mean $\pm$ SD = 3.9

± 1.3 mg/dL) than JEV IgM-negative AMES cases (Table 5). Among JEV IgM-positive cases, there were significant trends in types of CNS manifestations by age: among older cases, higher proportions experienced consciousness disorders (63–81%), whereas among the youngest cases (in children < 5 years of age), convulsions were more common (74–86%).

Among laboratory-confirmed JE cases, those with initial clinical JE diagnosis had higher proportions with lethargy (87%), drowsiness (72%), consciousness disorders (83%), nausea and vomiting, and stiff neck (59%) than cases with other clinical diagnoses ($P < 0.05$) (Table 5). There were no significant differences for other indicators.

DISCUSSION

This is the first effort in China to perform population-based surveillance for acute meningitis and encephalitis supported by comprehensive laboratory testing for selected vaccine-preventable etiologies. During the two years from September 2006 through September 2008, we identified 4,513 AMES cases, with an overall incidence of approximately 10 cases/100,000 residents of the four prefectures. Among the AMES cases reported by sentinel hospitals, 9.3% were laboratory confirmed as JE. The incidence and epidemiology of laboratory-confirmed JE varied markedly by prefecture, with predominance in young children in Guigang Prefecture and frequent occurrence in adults in the other three prefectures. The adjusted JE incidence rates were higher than those obtained from the crude surveillance results and varied almost 20-fold (from 0.08/100,000 persons in Shijiazhuang to 1.58/100,000 persons in Guigang). Overall, 70.4% (150 of 213) of the JEV IgM-positive cases had other, non-JE initial diagnoses. Among the 213 JEV IgM-positive cases, the common clinical features were typical of those of other studies, with a high degree of fever and mental changes, and percentages with lethargy, drowsiness, consciousness disorders, and convulsions were high.

Japanese encephalitis virus, *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type b are serious but vaccine-preventable causes of meningitis and encephalitis in China. Previously, surveillance for the bacterial meningitis and viral encephalitis has been conducted separately, with only JE and *N. meningitidis*

TABLE 4
Initial clinical diagnoses of JEV IgM-positive cases in sentinel hospitals, People's Republic of China*

Initial diagnosis	Total AMES cases, no. (%)	No. tested	JEV IgM+ cases
			No. (% tested)
Japanese encephalitis	117 (4.2)	112	63 (56.3)
Viral encephalitis	914 (32.5)	709	49 (6.9)
Viral meningitis	157 (5.6)	129	7 (5.4)
Viral meningoencephalitis	123 (4.4)	105	9 (8.6)
Other encephalitis	645 (22.9)	544	49 (9.0)
Meningococcal meningitis	13 (0.5)	12	0
Purulent meningitis	190 (6.7)	163	7 (4.3)
Cerebrospinal meningitis	5 (0.2)	5	0
TB meningitis	101 (3.6)	73	1 (1.4)
TB meningoencephalitis	26 (0.9)	19	0
Other meningitis	44 (1.6)	33	0
Other diagnosis	466 (16.6)	378	27 (7.1)
Missing	14 (0.5)	12	1 (8.3)
Total	2,815 (100)	2,294	213 (9.30)

* JEV = Japanese encephalitis virus; AMES = acute meningitis and encephalitis syndrome; TB = tuberculosis.

TABLE 5

Comparison of clinical symptoms and signs between cases with JEV IgM-positive or -negative results and between the cases with initial JE diagnosis and those with other initial diagnoses, People's Republic of China*

Characteristic	JE tested cases			Tested JE-positive cases		
	JEV IgM+ (n = 213)	JEV IgM- (n = 553)	P	Initial JE diagnosis (n = 63)	Other initial diagnosis (n = 149)	P
Fever	205 (97.2)	437 (80.9)	0	63 (100)	142 (95.9)	0.183†
Degree of fever‡	128 (62.1)	180 (40.9)	0	44 (69.8)	84 (58.8)	0.130
Headache	81 (62.8)	359 (78.2)	0	23 (57.5)	58 (65.2)	0.405
Diarrhea	14 (6.7)	38 (7.2)	0.795	1 (1.6)	13 (8.8)	0.057
Nausea	69 (36.1)	235 (46.7)	0.012	28 (48.3)	41 (30.8)	0.021
Vomiting	97 (46.0)	269 (50.4)	0.279	38 (60.3)	59 (39.9)	0.006
Projectile vomiting	9 (9.9)	33 (12.1)	0.563	6 (17.6)	3 (5.3)	0.056
Lethargy	154 (75.1)	333 (62.5)	0.001	53 (86.9)	101 (70.1)	0.011
Drowsiness	111 (55.2)	169 (31.8)	0	41 (71.9)	70 (48.6)	0.003
Consciousness disorder	117 (56.8)	140 (26.2)	0	52 (82.5)	65 (45.5)	0
Convulsion	133 (65.2)	123 (23.3)	0	39 (66.1)	94 (64.8)	0.862
Stiff neck	92 (44.4)	164 (30.9)	0	36 (59.0)	56 (38.4)	0.006
Opisthotonos	12 (5.9)	12 (2.3)	0.014	5 (8.5)	7 (4.8)	0.310
Meningeal irritation	75 (36.42)	141 (26.7)	0.011	29 (46.0)	46 (32.2)	0.057
Skin petechiae	11 (5.4)	21 (4.0)	0.409	3 (4.9)	8 (5.6)	0.853
CSF leukocyte count, cell/mm ³	69.1 ± 98.3	94.6 ± 182.7	0.056§			
CSF glucose, mg/dL	3.9 ± 1.3	3.6 ± 4.5	0§			
CSF protein, mg/dL	0.5 ± 0.5	0.7 ± 1.0	0.429§			
Died	7 (3.3)	6 (1.1)	0.055†	2 (3.2)	5 (3.4)	0.946

* Values are no. (%) or mean ± SD. JEV = Japanese encephalitis virus; CSF = cerebrospinal fluid. Unknown data were not included in calculations of statistical significance. JE IgM—includes only persons from whom serum was obtained ≥ 7 days after onset of illness.

† By Fisher test.

‡ No. of cases persons with a temperature > 39°C.

§ By Kruskal-Wallis test.

(epidemic meningitis) being reportable diseases. In our study, the incidence of AMES cases in the four prefectures was approximately 10/100,000, exceeding the current measles reporting incidence rate in China.⁸ Because the AMES cases had severe nervous system changes,^{9,10} the disease burden, including sequelae, is substantial.¹¹

Japanese encephalitis was highly endemic in China through the 1970s. However, because of the expanded use of JE vaccine, there has been a marked decrease in incidence and substantial changes in the epidemiology and geographic distribution of JE cases.^{12,13} Among the four prefectures included in this project, the estimated JE incidence and age distribution of cases varied substantially. These differences may be caused by several factors, including variations in ecology, population characteristics, and use of JE vaccine. Only Shandong Province had integrated JE vaccine into the provincial EPI in 1986 and it achieved systematic vaccination of children during this period. This finding may be a key factor in the older age distribution of cases in this prefecture, compared with Guangxi Province, another province that was highly endemic for JE, which had not integrated JE vaccination into its EPI, and which had a classical JE age distribution of cases, mainly in young children. Reasons for lower incidence in Yichang and Shijiazhuang were not identified and were beyond the scope of this project. Nevertheless, although there has been a substantial decrease in JE incidence nationally, the incidence remains relatively high in some provinces, and it still causes a great disease burden in the disease-epidemic areas.^{7,14}

Currently, JE and epidemic meningitis surveillance are passive and based on clinical diagnosis, with limited laboratory confirmation. In our study, the percentage of initial clinically diagnosed JE cases was only 30% (63 of 213) among all JEV IgM-positive cases. Most of the JE-positive cases had other initial clinical diagnoses, such as viral encephalitis, other encephalitis, or other diagnosis. The lower percentage of

initial JE diagnosed AMES cases demonstrates that clinical doctors prefer to give a diagnosis of viral encephalitis or other encephalitis rather than JE if there are no supporting laboratory results. Overall, the proportion of AMES cases caused by JE (9.3%) was lower than another acute encephalitis surveillance study in China, in which 30% of acute encephalitis surveillance cases were caused by JE.¹⁵ However, our study included all clinically diagnosed meningitis and encephalitis cases. Overall, the age and sex distribution of confirmed JE cases did not change with expanded laboratory testing. These findings point to a need to increase suspicion of JEV as a cause of AMES, particularly during the local JEV transmission season, and increase the use of laboratory testing to determine the etiology of AMES cases.

Among JEV IgM-positive cases, the percentage of the cases having fever, high degree of fever, mental changes, such as lethargy, drowsiness, consciousness disorders and convulsions, were significantly higher than that in the JEV IgM-negative AMES cases. One study reported that convulsions occurred in up to 85% of children and 10% of adults with confirmed JE cases.¹⁶ We had similar results in our study, with convulsion occurring in 86% of children < 2 years of age and 38% of adults ≥ 45 years of age. Our results are consistent with those of other studies in China, which have shown that JE cases had substantial central nervous system symptoms, which result in substantial sequelae, including long-term disability.¹¹

Among JE-positive cases, comparison of cases initially diagnosed as JE by the clinician and those with different initial diagnoses suggested that cases with an initial diagnosis of JE had more severe clinical symptoms or showed the typical JE clinical manifestations, which demonstrated that the clinical doctors recognize and diagnose typical or severe JE cases and preferred to diagnose the mild JE cases as other viral or other encephalitis.

This study had several limitations. First, most specimens were obtained within the first week after illness onset, and a second convalescent-phase specimen was obtained in < 2% of cases. Studies have shown that IgM elicited in JEV infection can take up to 10 days to reach levels detectable by the JEV IgM ELISA, although generally it is detectable by 5–7 days.¹⁷ In our study, among the clinically diagnosed and laboratory-confirmed JE cases, most specimens (88%) were obtained ≥ 3 days after onset of illness, whereas among clinically diagnosed JE cases with JEV IgM-negative results, 41% of the specimens were collected within 2 days of illness onset. These specimens may have had false-negative results, which suggests that additional JE cases would have been identified if we had obtained follow-up specimens at least 7 days after illness onset or convalescent-phase specimens. Similarly, during the JEV transmission season, among 692 AMES cases with JEV IgM-negative results, 38% of the specimens were collected within 3 days of illness onset, possibly before IgM levels increased to detectable levels. Thus, if we had been able to obtain follow-up specimens, we would have likely found additional positive JE cases by serologic testing, which would have indicated a more accurate disease burden. Detection of viral RNA by reverse transcription–polymerase chain reaction has also been shown to increase sensitivity in CSF specimens obtained within the first few days of illness.¹⁸ However, we were not able to carry out molecular testing because of storage and transport conditions and the varying capacities of the laboratories to conduct molecular testing. Thus, the true burden of JE is likely underestimated.

Specificity of serologic assays is also a problem in JE diagnosis because antibodies elicited in flavivirus infections are highly cross-reactive to heterologous flavivirus antigens in serologic assays. This cross-reactivity can result in false-positive results. Flavivirus serologic cross-reactivity occurs particularly in areas where multiple flaviviruses, such as JE and dengue viruses, may be co-circulating, and where the patients have been exposed to multiple flaviviruses. Dengue outbreaks occurred in Guangxi Province in the 1990s and more recently dengue virus has been isolated in areas along the Guizhou–Guangxi border.^{19,20} Although it is rare, dengue virus neurologic infections in AMES patients have been reported.²¹ The possibility that some of the JEV IgM-positive specimens are false-positive results for JE, and are instead dengue virus infections, cannot be ruled out, because only JEV IgM testing was conducted. However, given the low endemicity of dengue in China, the probability of AMES cases with dengue virus infection is low.

The Beixa test kit we used was chosen for all the participating laboratories because of its advantages, such as its availability and affordability, and its accuracy and reproducibility when compared with a reference PanBio JE IgM test (unpublished data). However, sensitivity and specificity of the Beixa kit has not been determined, as for other JEV IgM ELISA kits.^{22,23}

We did not enroll all hospitals in the prefecture and surrounding areas and some resident cases may have sought care outside our surveillance area. Finally, there may have been some differences between AMES cases seeking care in sentinel and non-sentinel hospitals, resulting in errors of estimating overall disease incidence. However, in our study, most of the JE-confirmed cases were living in the rural areas. Thus, we believe that these limitations are relatively small given the overall size and importance of the major cities within the

study prefectures, which would attract almost all persons with cases of disease in the surrounding area.

This project has provided important insights into the varying incidence and epidemiology of JE in China. From this study, it was clear that surveillance with laboratory confirmation is important for estimation of the disease burden and accurate diagnosis of JE cases. Based on this study, we suggest that JE surveillance and control should be enhanced by wider use of laboratory testing to confirm JE as a cause of AMES cases, especially during the local JEV transmission season. In addition, clinical doctors should consider all viral cases, including typical severe cases and mild cases, as possible JEV infections. Data from enhanced JE surveillance should be used to further refine JE prevention strategies, according to the different areas that have different JE epidemic characteristics, and some areas should consider the need for vaccination of older age groups.

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