Recurrence of Japanese Encephalitis Epidemic in Wuhan, China, 2009–2010

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Abstract

**Background:** Japanese encephalitis (JE) was once epidemic in most areas of China, including Wuhan, a city located in the central part of China. The incidence of JE dramatically decreased due to nationwide immunization with the live attenuated JE virus (JEV) vaccine, and no JE cases were reported during 2005–2008 in Wuhan. In 2009 and 2010, 31 JE cases reoccurred in this area. In this study, we investigated the causes of JE recurrence.

**Methods and Findings:** All JE cases were laboratory-confirmed by detecting the JEV-specific IgM antibody with an IgM-capture enzyme-linked immunosorbent assay (ELISA). All patients were children between 2 months and 9 years of age with a median age of 2 years. Of the 31 cases, 9 had received one or two doses of the JEV vaccine, 11 had not been immunized previously with the JEV vaccine, and 11 had an unclear immunization history. Through reverse transcription polymerase chain reaction (RT-PCR), sequencing, and phylogenetic analysis, two new strains of JEV were isolated from Culex tritaeniorhynchus and identified as genotype 1 JEV, rather than genotype 3, which circulated in this area previously.

**Conclusions:** Vaccine failure or missed vaccination may have caused JE recurrence. Local centers for disease control and prevention need to improve immunization coverage, and the efficacy of the JE vaccine needs to be reevaluated in a population at risk for disease.

Introduction

Japanese encephalitis (JE) is an acute epidemic disease of the central nervous system caused by infection with the Japanese encephalitis virus (JEV), which primarily affects children and adolescents [1,2]. It was recently estimated by the World Health Organization (WHO) that the annual case frequency of JE is 67,897 in JE-endemic areas, most of whom are children under 15 years old. The case mortality rate is 20–30%, and neurologic or psychiatric sequela occurs in 30–50% of survivors [2–5]. JE occurs throughout most of Asia and parts of the western Pacific [6,7,8].

Extensive JE vaccination programs have been implemented in JE endemic countries. Asian countries, like Japan and Korea, which have had major epidemics in the past, have already controlled JE through extensive JE vaccination programs. However, JE is still a life-threatening disease to people living in endemic areas in developing countries, mainly due to the difficulties of controlling the JE vector and amplifier [9]. In the 1990s, outbreaks were reported in Australia and on the island of Saipan. In both, mosquito vectors were believed to be involved [10,11].

JEV is an arthropod-borne virus (arbovirus) that is transmitted in an enzootic cycle between mosquitoes and amplifying vertebrate hosts, mainly pigs and wading birds [12,13,14]. JEV is the most common pathogen leading to viral encephalitis in Asia. JEV strains have been divided into five genotypes, and genotypes 1 and 3 are distributed widely in Asia, including China, Japan, Korea, India, Vietnam, and the Philippines [15].

JEV cases have been reported in most provinces of China except Xinjiang Uygur Autonomous, and Qinghai Province [1,16]. Since an extensive JE vaccination program started for children in the 1970s, the number of JE cases has significantly decreased nationwide, from 174,932 cases of morbidity in 1971 to 5,097 cases in 2005 [16]. However, outbreaks still occur in some provinces, especially in the middle and western areas of China [16,17]. Here, we report that 31 JE cases occurred from 2009 to 2010 in Wuhan, which is located in the central part of China and is the capital of Hubei Province.

In Wuhan, the incidence rate of JE dramatically decreased in the early 1990s (Figure 1), when a booster JE vaccination campaign began to immunize children under 15 years old in rural areas with live attenuated vaccine (SA14-14-2, manufactured...
by Chengdu Institute of Biological Products, China) in April every year at their own expense [18, 19]. Between 2005 and 2008, no JE cases were reported. In the present study, we collected epidemiological data from JE patients, piglets, and mosquitoes in the areas of confirmed JE cases to explore the possible causes for the recurrence of JE in the Wuhan area.

Materials and Methods

Ethics statement

All the experiments involving animals and humans were approved by the Ethics Committee of the Medical Research Council of Wuhan. Signed informed consents were obtained from parents prior to participation.

Subjects

In 2009 and 2010, all suspected JE cases reported to the Chinese Disease Reporting Information System (CDRIS) in Wuhan were further investigated by the Wuhan Centers for Disease Control and Prevention (CDC) according to a WHO-recommended JE surveillance project [20]. Patients and caregivers were interviewed, medical records were checked, sera for JEV-specific antibody testing were collected, and other epidemiological data, such as history of JE vaccination (recorded date and vaccination dose were confirmed by reviewing immunization certificates) and travel history before disease onset, were collected. A suspected JE case is one that meets the clinical case definition for viral encephalitis syndrome, which is defined as a person with acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk). A laboratory-confirmed case is one in which the JE virus-specific IgM antibody is detected from a single serum sample from the suspected case with an IgM-capture ELISA [20], which is the recommended method for laboratory confirmation of a JEV infection by the WHO. In addition, other serum testing was done to rule out infection due to a cross-reacting flavivirus-like dengue virus.

Serological surveillance on piglets

Blood samples for serological studies were also collected from piglets (pigs aged less than 3 months) on a farm in Dongxihu District of Wuhan (near the areas of JE cases) from April to October 2009. The antibody response (i.e. IgM and IgG) to JEV was detected by JE Detect™ IgG/IgM antibody capture ELISA kits (Inbios International, Inc., Seattle, WA, USA).

Mosquito monitoring

Mosquitoes were captured with light traps hung near pigpens close to JE case’s houses. All mosquitoes captured were anesthetized by carbon dioxide and then grouped according to their type and sex. Mosquitoes were classified into three groups (Culex, Aedes, Anophele) and stored in tubes with 50–100 mosquitoes as a pool based on their classification. The tubes were kept frozen in a liquid nitrogen tank until virus isolation.

Cell line and cell culture

BHK-21, a baby hamster kidney derived fibroblast cell line, obtained from the American Type Culture Collection (ATCC CCL10), was a gift kindly provided by the College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China. The BHK cells were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 2 mM-L-glutamine (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Invitrogen, Melbourne, Australia), 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Grand Island, NY, USA) in T-25 flasks with 5% CO₂ at 37°C.

Figure 1. Incidence rate of Japanese encephalitis (JE) in Wuhan, China (1992–2004). From 1992 to 2004, the incidence rate of JE decreased in Wuhan, China. doi:10.1371/journal.pone.0052687.g001
Virus isolation

Viruses were isolated as described previously [21,22]. Briefly, 2 ml of DMEM was added into each tube containing the mosquito pool, followed by homogenizing the mixture in a glass homogenizer on ice. The homogenate was collected and centrifuged at 3,000 rpm for 15 min. The supernatant (200 µl) was filtered through a 0.2 µm syringe and added to BHK-21 cells in a 6-well culture plate containing 2% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. After the cells were passaged at least three times, the supernatants were harvested after 5 days of culture.

Virus identification

Total RNA was extracted from supernatants of infected BHK-21 cells using an Rneasy Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed with a one step RT-PCR kit (Qiagen, Hilden, Germany) to amplify a 1541 bp JE E gene fragment (JEEF). The primer sequences used for RT-PCR were as follows: forward primer JEEF 5’-GGTGGTTCGGGCTTTACAGTTT-3’ and reverse primer JEER 5’-GATGTCATTGGCCACAGCCGT-3’. The RT-PCR conditions were as follows: cDNA synthesis at 50°C for 30 min, pre-denaturation at 94°C for 15 min, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 90 s. The positive PCR products were sent to Sangon Biotech Co. Ltd. (Shanghai, China) for sequencing with bidirectional primer measuring communicability.

Multiple alignments and phylogenetic analysis

A total of 30 JEV strains, including two new JEV strains isolated from Culex tritaeniorhynchus near the JE cases’ homes and the live attenuated vaccine virus strain SA14-14-2 were analyzed. In addition, Murray Valley encephalitis virus (MVEV) was analyzed as an out group strain. The nucleotide sequences of all strains were obtained from Genbank, along with their places of isolation, years of isolation, and accession numbers. The E gene and deduced amino acid sequences of all 30 strains were aligned with ClustalX 1.83, Mega software version 4.0, and the phylogeny tree was constructed using the neighbor-joining (NJ) method [23]. Constructed NJ trees were analyzed with bootstrap of 1000 replicates using Kimura’s two-parameter method.

Data management and analysis

Data for all cases, vectors, and contacts were entered into Excel 2007 spreadsheets (Microsoft, USA). The analysis was conducted by filter functions and statistical programs in this software. The Epi-Info (version 3.5.1, CDC, Atlanta, GA, USA) program was also used for statistical analysis. Double original data entries and computer printouts were repeatedly performed to verify data quality.

Results

Epidemiological study of JE cases

Wuhan Children’s Hospital, the only hospital accredited for diagnosing JE in Wuhan, reported all suspected JE cases of Wuhan residents during 2009 to 2010. A total of 31 cases were laboratory-confirmed JE. The serum did not have cross-reactivity to dengue virus. Eleven JE cases had disease onset in 2009 and 20 had disease onset in 2010; the incidence rates were 0.14/100,000 in 2009 and 0.25/100,000 in 2010 (chi-squared test, p > 0.05). With no travel history within two weeks before onset, all JE cases went to Wuhan Children’s Hospital for treatment. Most of the cases demonstrated typical encephalitis symptoms, i.e. unconsciousness, lethargy, and mental clouding. After treatment, 27 patients recovered, 3 patients presented with dementia symptoms, and 1 patient died. The prevalence of JEV was slightly higher in males than in females with a M: F ratio of 1.38:1 (18/13). All patients were children between 2 months and 9 years of age with a median age of 2 years. Twenty-two (70.97%) patients lived at home, five (16.13%) were in day-care centres, and four (12.90%) were students. All of the JE cases presented onset of the disease between July and August, except for one in May. Nine patients received a JE vaccination before onset. Of these, three received two doses of the vaccine and six received one dose of the vaccine. Eleven (35.40%) cases had not been immunized, and the JE vaccination status of the remaining 11 (35.40%) cases was unknown. The details are presented in Table 1. Information regarding vaccination dates, date of onset, and outcome of the disease is included in Table S1.

In 2009, JE cases occurred in four different districts of Wuhan, and 72.73% of JE patients resided in Huang Pi District, a rural area of Wuhan. In 2010, JE cases were widely distributed across nine districts of Wuhan, the patients either were children living in rural areas or were children who left their hometown and lived in a place without a local registration card (migrant children). The details are shown in Table 2.

Serological analysis for piglets

Serum samples were collected from 209 piglets at farms in Dongxiu District and detected for JEV antibody. As indicated in Table 3, the serum prevalence of JEV-specific antibodies was seasonally related; it was 100% JEV antibody positive in July and August, but it had a lower prevalence in April (66.67%) and October (37.93%).

Table 1. Characteristics of Japanese encephalitis cases in Wuhan, China (2009–2010).

| Characteristic                  | No. | %    |
|--------------------------------|-----|------|
| No. of cases                   | 31  |      |
| Gender                         |     |      |
| Male                           | 18  | 58.06|
| Female                         | 13  | 41.94|
| Age group                      |     |      |
| <8 months                      | 3   | 9.68 |
| 8 months-1 year                | 5   | 16.13|
| 2–5 years                      | 17  | 54.84|
| 6–9 years                      | 6   | 19.35|
| Living status                  |     |      |
| at home                        | 22  | 70.97|
| in day-care center             | 5   | 16.13|
| at school                      | 4   | 12.90|
| Immunization status            |     |      |
| 2 doses                        | 3   | 9.68 |
| 1 dose                         | 6   | 19.36|
| 0 doses                        | 11  | 35.48|
| Unknown                        | 11  | 35.48|
| Antibody results               |     |      |
| IgM positive                   | 31  | 100  |
| IgG positive                   | 7   | 22.58|
| Outcome of the disease         |     |      |
| Recovery                       | 27  | 87.10|
| Dementia                       | 3   | 9.67 |
| Death                          | 1   | 3.23 |

doi:10.1371/journal.pone.0052687.t001
Mosquito monitoring

A total of 84,089 adult mosquitoes were trapped nearby JE case’s houses. *Culex tritaeniorhynchus* was the major type of mosquito (90.77%). Some of the mosquitoes belonged to *Culex pipiens fatigans* (4.22%) and to *Anopheles hyrcanus sinensis* (3.56%). The details are shown in Table 4.

Virus identification, multiple alignments, and phylogenetic analysis

Two new strains of the JE virus were isolated and identified from *Culex tritaeniorhynchus*. They belonged to genotype 1. No JE virus strains were isolated from other types of mosquitoes. These two new JE virus strains were named as WHJX09-09 (Genbank accession number HQ437283) and WHJX09-10 (Genbank accession number HQ538843). The phylogenetic analysis is shown in Figure 2. The homology of the two new JE virus strains was 98.90% in the nucleotide sequences and 100% in the deduced amino acid sequences. Comparing the two new JE virus strains to live attenuated vaccine strain SA14-14-2 in the E gene, the homology of the nucleotide sequence was 87.93% (WHJX09-09) and 88.33% (WHJX09-10), respectively, and the homology of

Table 2. Area distribution of Japanese encephalitis cases in Wuhan, China (2009–2010).

| District     | 2009 | 2010 | Total |
|--------------|------|------|-------|
|              | No.  | %    | No.  | %    | No.  | %    |
| Huang pi*    | 8    | 72.73| 4    | 20.00| 12   | 38.71|
| Xin zhou*    | 0    | 0    | 4    | 20.00| 4    | 12.90|
| Hong san*    | 1    | 9.09 | 3    | 15.00| 4    | 12.90|
| Jiang xia*   | 1    | 9.09 | 3    | 15.00| 4    | 12.90|
| Jiang an     | 1    | 9.09 | 2    | 10.00| 3    | 9.67 |
| Han yang     | 0    | 0    | 1    | 5.00 | 1    | 3.23 |
| Dong xihu*   | 0    | 0    | 1    | 5.00 | 1    | 3.23 |
| Han nan*     | 0    | 0    | 1    | 5.00 | 1    | 3.23 |
| Cai dian*    | 0    | 0    | 1    | 5.00 | 1    | 3.23 |
| Total        | 11   | 100  | 20   | 100  | 31   | 100  |

Rural areas are indicated by an asterisk (*).

doi:10.1371/journal.pone.0052687.t002

Table 3. Prevalence of neutralizing antibodies to Japanese encephalitis virus among piglets in Wuhan, China, 2009.

| Month  | No. of samples | No. JEVAb* (+) | % JEVAb (+) |
|--------|----------------|----------------|-------------|
| April  | 30             | 20             | 66.67       |
| May    | 30             | 25             | 83.33       |
| June   | 30             | 24             | 80.00       |
| July   | 30             | 30             | 100         |
| August | 30             | 30             | 100         |
| September | 30           | 21             | 70.00       |
| October| 29             | 11             | 37.93       |
| Total  | 209            | 161            | 77.03       |

*JEV Ab: Japanese Encephalitis Virus Antibody.

doi:10.1371/journal.pone.0052687.t003

Table 4. Classification of adult mosquitoes trapped nearby Japanese encephalitis cases’ homes in Wuhan, China, 2009–2010.

| Category                                                                 | No. | %   | No. | %   | No. | %   |
|--------------------------------------------------------------------------|-----|-----|-----|-----|-----|-----|
| *Culex tritaeniorhynchus*                                                | 76327| 66.67|     |     |     |     |
| *Culex pipiens fatigans*                                                 | 3547 | 4.22 |     |     |     |     |
| *Anopheles hyrcanus sinensis*                                            | 2996 | 25.71|     |     |     |     |
| *Aedes armigerus*                                                        | 853  | 1.68 |     |     |     |     |
| *Anopheles albofasciatus*                                                | 101  | 0.20 |     |     |     |     |
| *Aedes aegypti*                                                          | 159  | 0.20 |     |     |     |     |
| *Aedes annulipennis*                                                     | 11   | 0.05 |     |     |     |     |
| *Mansonia uniformis*                                                     | 8    | 0.04 |     |     |     |     |
| *Culex bitaeniorhynchus*                                                 | 159  | 0.20 |     |     |     |     |
| *Aedes vexans*                                                           | 11   | 0.05 |     |     |     |     |
| *Mansonia uniformis*                                                     | 11   | 0.05 |     |     |     |     |
| *Aedes aegypti*                                                          | 8    | 0.04 |     |     |     |     |
| *Culex bitaeniorhynchus*                                                 | 159  | 0.20 |     |     |     |     |
| *Aedes vexans*                                                           | 11   | 0.05 |     |     |     |     |
| *Mansonia uniformis*                                                     | 8    | 0.04 |     |     |     |     |
| *Aedes aegypti*                                                          | 11   | 0.05 |     |     |     |     |
| *Culex bitaeniorhynchus*                                                 | 159  | 0.20 |     |     |     |     |
| *Aedes vexans*                                                           | 11   | 0.05 |     |     |     |     |
| *Mansonia uniformis*                                                     | 8    | 0.04 |     |     |     |     |
| *Aedes aegypti*                                                          | 11   | 0.05 |     |     |     |     |

doi:10.1371/journal.pone.0052687.t004
amino acids was 96.90% (a total of 15 amino acids were different from SA14-14-2). The mutated amino acids were located in the E gene antigen determinants, domain I (E138, 176, and 177), domain II (E57, 107, 129, 222, 244, 264, and 279), and domain III (E315, 327, and 366). The details are shown in Table 5.

Discussion

In the present study, we reported the recurrence of JE in Wuhan, China, during 2009–2010. A total of 31 cases were laboratory-confirmed JE, 11 cases were onset in 2009, and 20 cases occurred in 2010. We investigated the patients’ immuniza-
Culex mosquitoes, especially in the southern region of China, are suitable for mosquitoes to grow and reproduce. JEV is maintained in a natural cycle of transmission involving mosquitoes, which then transmit JEV to humans. The annual average temperature is 15.8–17.5°C, and the average annual rainfall is 1269 mm. The geographical and environmental conditions are very suitable for mosquitoes to grow and reproduce. JEV is maintained in a natural cycle of transmission involving mosquitoes, which then transmit JEV to humans. Culex mosquitoes, especially *Cx. tritaeniorhynchus*, are the principal vector for both zoonotic and human JEV transmission [12,13,24,25,26]. The majority of mosquitoes trapped from JE epidemic focus of JE.

In Wuhan, JE live attenuated vaccine (LAV) of the SA14-14-2 strain was used for preventing disease. Studies have shown that this vaccine is safe, well tolerated, and highly immunogenic, and a single dose of JE vaccine can provide strong protection against JE [27,28]. However, there are no mutations in the E protein domain III residues (E307–E309, E327–E333, and E386–E390). Previous studies have shown that these three domain III residues of E protein could form a novel cis-proline turn structure and are important in eliciting JEV-specific neutralizing antibodies [33]. Based on this information, it seems that the current vaccine still possesses the ability to protect children from JE infection, at least in theory. The nine children who had received the JE vaccine but still acquired infection may be associated with a change in the immune response, since the mutation of new isolates in Wuhan affected the antigen determinants of the JEV envelope protein. A similar JE case was also reported from Yunnan Province, where genotype 1 JEV was obtained from the cerebrospinal fluid sample of a 2-year-old boy who had been immunized with one dose of JE live attenuated vaccine [1].

To understand if these two new JEV strains led to the vaccine losing its protective efficacy and could be the cause of the JE recurrence was associated with either failure of vaccination or missed vaccination.

In Wuhan, JE live attenuated vaccine (LAV) of the SA14-14-2 virus was used for preventing disease. Studies have shown that this vaccine is safe, well tolerated, and highly immunogenic, and a single dose of JE vaccine can provide strong protection against JE [27,28]. In addition, a test in mice for evaluating the protective efficacy of this vaccine has shown that the protection efficacy against intraperitoneal challenge with 16 virus strains (both genotype 1 and genotype 3) was 80–100% [29]. There are two JE virus strains circulating in China: genotype 3 and genotype 1. Genotype 1 JEV was first isolated from mosquitoes in Yunnan Province in 1979 [30], and it was also detected from mosquitoes in the provinces of Liaoning, Heilongjiang, Fujian, Hunan, and Shanxi [30,31,32]. In the Wuhan area, a genotype 3 JEV strain was isolated from mosquitoes in 1988 (Genbank accession number AV849939), and no genotype 1 JEV strain has been reported. In the present study, we identified two new JEV strains for the first time, and both of these strains belong to genotype 1.

| Strain            | E56 | E107 | E129 | E138 | E176 | E177 | E222 | E244 | E264 | E279 | E315 | E327 | E366 | E439 | E447 |
|-------------------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| SA14-14-2         | Val | Phe  | Thr  | Lys  | Val  | Ala  | Ala  | Gly  | His  | Met  | Val  | Ser  | Ala  | Arg  | Asp  |
| WHJX09-09         | Ile | Leu  | Met  | Glu  | Ile  | Thr  | Ser  | Glu  | Gln  | Lys  | Ala  | Thr  | Ser  | Lys  | Gly  |
| WHJX10-09         | Ile | Leu  | Met  | Glu  | Ile  | Thr  | Ser  | Glu  | Gln  | Lys  | Ala  | Thr  | Ser  | Lys  | Gly  |

Table 5. Comparison of the SA14-14-2 strain with two newly isolated JEV strains, WHJX09-09 and WHJX09-10, at the amino acid level of E protein.

doi:10.1371/journal.pone.0052687.t005

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improving the JE immunization coverage rate in poor and remote areas, the local CDC still needs to organize a mass campaign against JE, and more seminars need to be provided for caregivers to better understand JE. In addition, new JE vaccines may need to be developed against new strains of JEV.

Supporting Information

Table S1  The status of vaccination and the outcome of the disease.

References

1. Zhang JS, Zhao QM, Guo XF, Zuo SQ, Cheng JX, et al. (2011) Isolation and genetic characteristics of human genotype I Japanese encephalitis virus, China, 2009. PLoS One 6: e16418.

2. Grau LC, Susan LH, Marc F, Julie AJ, Charles IH, et al. (2011) Estimated global incidence of Japanese encephalitis: a systematic review. Bulletin of the World Health Organization 89: 766–774E.

3. Fischer M, Lindsey N, Staples JE, Hills S (2010) Japanese encephalitis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 59: 1–27.

4. Solomon T (2006) Control of Japanese encephalitis–within our grasp? N Engl J Med 355: 869–871.

5. Ghosh D, Basu A (2009) Japanese encephalitis-a pathological and clinical perspective. PLoS Negl Trop Dis 3: e187.

6. Sohn YM (2000) Japanese encephalitis immunization in South Korea: past, present, and future. Emerg Infect Dis 6: 17–24.

7. Halstead SB (1998) Vaccines for Japanese encephalitis. Lancet 348: 341.

8. Wu YC, Huang YS, Chien LJ, Lia TL, Yen YY, et al. (1999) The epidemiology of Japanese encephalitis on Taiwan during 1966–1997. Am J Trop Med Hyg 61: 78–84.

9. Akiba T, Osaka K, Tang S, Nakayama M, Yamamoto A, et al. (2001) Analysis of Japanese encephalitis epidemic in Western Nepal in 1997. Epidemiol Infect 126: 81–88.

10. Hama JN, Ritchie SA, Phillips DA, Shield J, Bailey MC, et al. (1996) An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. Med J Aust 165: 236–260.

11. Paul WS, Moore PS, Karabatsos N, Flood SP, Yamada S, et al. (1993) Outbreak of Japanese encephalitis on the island of Saipan, 1990. J Infect Dis 167: 1053–1058.

12. Rosen L (1986) The natural history of Japanese encephalitis virus. Annu Rev Microbiol 40: 395–414.

13. Vaughn DW, Hoke CH Jr (1992) The epidemiology of Japanese encephalitis: prospects for prevention. Epidemiol Rev 14: 197–221.

14. Buescher EL, Scherer WF (1958) Ecologic studies of Japanese encephalitis virus in Japan. IX. Epidemiologic correlations and conclusions. Am J Trop Hyg 8: 719–722.

15. Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ, et al. (2003) Origin and evolution of Japanese encephalitis virus in southeast Asia. J Virol 77: 3091–3098.

16. Wang H, Li Y, Liang X, Liang G (2009) Japanese encephalitis in mainland china. Jpn J Infect Dis 62: 331–336.

17. Wang LH, Fu SH, Wang HY, Liang XF, Cheng JX, et al. (2007) Japanese encephalitis outbreak, Yuncheng, China, 2006. Emerg Infect Dis 13: 1123–1129.

18. Hemnay S, Liu Z, Tsai TF, Strom BL, Wan GM, et al. (1996) Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. Lancet 347: 1583–1586.

19. Chen BH, Hu Q, Yu B, Liu PL, Gao L (2007) Epidemic character of Japanese B encephalitis during 1992–2006 in Wuhan[In Chinese]. Journal of Public Health and Preventive Medicine 18: 25–27.

20. World Health Organization (2006)WHO-recommended standards for surveillance of selected vaccine-preventable diseases. Available: http://www.who.int/vaccine-documents/DocsPDF06/843.pdf. Accessed 11 Oct 2011.

21. Ritchie SA, Phillips D, Broom A, Mackenzie J, Poirnier M, et al. (1997) Isolation of Japanese encephalitis virus from Culex annulirostris in Australia. Am J Trop Med Hyg 56: 80–84.

22. Johansen CA, van den Hurk AF, Ritchie SA, Zborowski P, Nisbet DJ, et al. (2000) Isolation of Japanese encephalitis virus from mosquitoes (Diptera: Culicidae) collected in the Western Province of Papua New Guinea, 1997–1998. Am J Trop Med Hyg 62: 631–638.

23. Nga PT, del Carmen Parquet M, Cuong VD, Ma SP, Hasebe F, et al. (2004) Shift in Japanese encephalitis virus (JEV) genotype circulating in northern Vietnam: implications for frequent introductions of JEV from Southeast Asia to East Asia. J Gen Virol 85: 1625–1631.

24. Eady TP, Nisalak A (2007) Japanese encephalitis virus: ecology and epidemiology. Curr Top Microbiol Immunol 267: 11–48.

25. Gresser I, Hardy JL, Hu SM, Scherer WF (1958) Factors influencing transmission of Japanese B encephalitis virus by a colonized strain of Culex tritaeniorhynchus Giles, from infected pigs and chicks to susceptible pigs and birds. Am J Trop Med Hyg 7: 365–373.

26. Keiser J, Maithe MF, Erlanger TE, Bos R, Tanner M, et al. (2005) Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. Acta Trop 95: 40–57.

27. Chotpittayasunondh T, Sohn YM, Yoksan S, Min J, Ohrr H (2011) Immunizing children aged 9 to 15 months with live attenuated SA14-14-2 Japanese encephalitis vaccine in Thailand. J Med Assoc Thai 94 Suppl 3: S195–203.

28. Sohn YM, Tandan JB, Yoksan S, Ji M, Ohrr H (2008) A 5-year follow-up of antibody response in children vaccinated with single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine: immunogenicity and anamnestic responses. Vaccine 26: 1638–1643.

29. Liu X, Yu Y, Li M, Liang G, Wang H, et al. (2011) Study on the protective efficacy of SA14-14-2 attenuated Japanese encephalitis against different JEV virus isolates circulating in the China. Vaccine 29: 2127–2130.

30. Wang HY, Tomohiko T, Fu SH, Sun XH, Zhang H, et al. (2007) Molecular epidemiological analysis of Japanese encephalitis virus in China. Journal of General Virology 88: 885–894.

31. Zhang S, Yin Z, Suraratdecha C, Liu X, Li Y, et al. (2011) Knowledge, attitudes and practices of caregivers regarding Japanese encephalitis in Shaxian Province, China. Public Health 125: 79–83.

32. Wang HY, Fu SH, Li XY, Song H, Min JG, et al. (2004) Isolation and identification of genotype I Japanese encephalitis virus in China. Chin J Microbiol Immunol 24: 483–499.

33. Wu SC, Lin CW (2001) Neutralizing peptide ligands selected from phage-displayed libraries mimic the conformational epitope on domain III of the Japanese encephalitis virus envelope protein. Virus Res 76: 59–69.

Acknowledgments

The authors would like to thank Wuhan Children’s Hospital, Dongxihu, Jiangxia, Huangpi, Caidian, Hanyang, Xinzhou, Jianggan, Hancun Centre for Disease Control and Prevention in Hubei Province, China for sample collection. The authors thank Sandra Clark for editing the manuscript, and specially appreciate Michael M. Englund for advice on manuscript writing.

Author Contributions

Conceived and designed the experiments: QH BC. Performed the experiments: BC ZZ JT YZ. Analyzed the data: BC X. Zhang. Contributed reagents/materials/analysis tools: ZZ JT YZ. Wrote the paper: QH BC X. Zheng ZZ.