Serological and molecular epidemiology of Japanese encephalitis virus infections in swine herds in China, 2006–2012

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Japanese encephalitis virus (JEV) is a mosquito-borne, zoonotic flavivirus causing viral encephalitis in humans and reproductive disorder in swine. JEV is prevalent throughout China in human; however, spatiotemporal analysis of JEV in Chinese swine herds has not been reported previously. Herein, we present serological and molecular epidemiological results and estimates of prevalence of JEV infections among swine herds in various regions of China. The results suggest that JEV infections are widespread and genotype I and III strains co-exist in the same regions. Therefore, there is an urgent need to monitor JEV infection status among swine herds in China.

Keywords: China, Japanese encephalitis virus, molecular epidemiology, serological detection, swine
also displays seasonal and geographical distribution changes; JEV infections appear 1 to 2 months earlier in southern China than in northern China, and new JEV infections occur 1 to 2 months earlier in swine than in humans. Therefore, by monitoring JEV epidemic trends in swine, relevant and timely information can be acquired and used to better predict JEV epidemics among humans, thereby promoting effective prevention and control of human JEV infections.

Previous nucleotide sequencing of the capsid/premembrane protein (C/PrM) and envelope (E) genes of JEV revealed five genotypes [3], and genetic analysis of JEV strains isolated from humans and mosquitoes showed that JEV genotypes I and III co-exist in China [3]. To uncover the status of JEV in swine in China, mosquito samples were collected and viruses isolated and sequenced. The results demonstrated that JEV genotypes I and III were prevalent in Chinese swine herds, and the JEV isolated from mosquitoes shared high homology with those isolated from humans and pigs [3-5].

To understand the genetic relationships among JEV strains isolated from pigs in China, 18 available JEV sequences were extracted from the NCBI database (National Center for Biotechnology Information, USA), and phylogenetic analysis based on the C/PrM and E nucleotide sequences was performed (Table 1). Of the 18 JEV strains isolated between 2006 and 2012 in China, most were isolated from aborted fetuses; however, a few were isolated from piglets with confirmed viral encephalitis. Our results indicated that 12 of the isolated strains belonged to genotype I and the remaining 6 strains were identified as genotype III, indicating that these two genotypes co-exist within the swine herds in China (Fig. 2). In China, the first JEV strain (AY849939), which belongs to genotype III, was isolated from swine in 1988 [2,3]. In 2007, a genotype I strain (FJ495189) was isolated from a pig herd in China [2]. Thereafter, several genotype I strains were isolated from pigs, indicating that JEV...
Table 1. Strains of Japanese encephalitis virus identified in GenBank and used in phylogenetic analysis of premembrane protein-coding genes

| Genotype | Accession No. | Strain | Source of virus | Collection year | Geographical origin |
|----------|---------------|--------|-----------------|-----------------|---------------------|
| I        | HQ893545      | SX09S-01 | Swine brain     | 2009            | China               |
| I        | HQ893551      | SX09S-02 | Swine brain     | 2009            | China               |
| I        | HQ893546      | SX09S-A  | Swine brain     | 2009            | China               |
| I        | HQ893547      | SX09S-B  | Swine brain     | 2009            | China               |
| I        | HQ893548      | SX09S-C  | Swine brain     | 2009            | China               |
| I        | HQ893549      | SX09S-E  | Swine brain     | 2009            | China               |
| I        | HQ893550      | SX09S-F  | Swine brain     | 2009            | China               |
| I        | AB241119      | Mie41   | Swine serum     | 2002            | Japan               |
| I        | GQ336810      | Henan-09-03 | Pig | 2009 | China |
| I        | FJ495189      | HEN0701 | Swine serum     | 2007            | China               |
| I        | GU556217      | JX61    | Pig Serum       | 2008            | China               |
| I        | FJ179364      | JX66    | Pig Serum       | 2008            | China               |
| I        | FJ179365      | JX67    | Pig Serum       | 2008            | China               |
| I        | AY316157      | KV1899  | Pig             | 2003*           | Korea               |
| I        | GQ902061      | B-1381-85 | Pig | 2009 | Thailand |
| II       | HQ223287      | JKT654  | Mosquito        | 1978            | Indonesia           |
| II       | AF217620      | FU      | NA              | 1995*           | Australia           |
| III      | KC915016      | GZ      | Swine brain     | 2010            | China               |
| III      | LF297916      | GZ09    | Pig seminal fluid | 2004 | China |
| III      | AY849939      | HW      | Swine           | 1988            | China               |
| III      | EF107523      | WHe     | Swine           | 2006            | China               |
| III      | GQ495003      | HLJ08-01 | Swine | 2008 | China |
| III      | GQ495000      | HLJ08-02 | Swine | 2008 | China |
| III      | GQ495001      | HLJ08-03 | Swine | 2008 | China |
| III      | JQ086762      | YUNNAN0901 | Mosquito | 2009 | China |
| III      | JQ086763      | YUNNAN0902 | Mosquito | 2009 | China |
| III      | LF297915      | GD01    | Pig aborted fetuses | 2009 | China |
| IV       | L42158        | JKT9092 | NA              | NA              | Indonesia           |
| IV       | AY184215      | JKT7003 | NA              | NA              | Indonesia           |
| IV       | AY184212      | JKT6468 | Mosquito        | 1981            | Indonesia           |
| V        | JF915894      | XZ0934  | Mosquito        | 2009            | China               |
| V        | HM596272      | Muar    | Homo sapiens brain | 1952 | Malaysia |

NA, not available. *Submitted year.

Genotype I is predominant in China [1,6-8].

In this study, we present the results of our serological and molecular epidemiological analyses of data obtained from previously reported JEV infections in swine herds in China. The results indicate that JEV infections are widespread throughout the sampled regions of China and confirm that genotype I and III strains co-exist at pig farms in the same region. The results indicate that increased surveillance of JEV among Chinese swine herds is warranted.

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Conflict of Interest

The authors declare no conflicts of interest.

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Fig. 2. Results of phylogenetic analysis of Japanese encephalitis virus strains isolated from Chinese swine herds based on assessment of the capsid/premembrane protein (C/PrM) nucleotide sequences. The multiple sequence alignments were obtained by using MEGA software (ver. 5.0; Molecular Evolutionary Genetics Analysis). The tree was constructed by applying the neighbor-joining method. Scale bar indicates number of nucleotide substitutions per site. Bootstrap confidence limits are shown at each node. The Dengue 1 virus (strain GZ2002) was used as an outlier group. Those strains (marked by ▲ or ●) were isolated and identified from pigs between 2006 and 2012 in China.

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