

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

Japanese encephalitis virus (JEV) is a zoonotic flavivirus that causes viral encephalitis in humans and reproductive disorder in swine in eastern and southern Asia [2]. In China, JEV strain P3 was isolated in 1949, and JE has been epidemic for succeeding 60 years; moreover, it has been reported that JEV is prevalent throughout China, including its presence in Tibet [4]. In 2015, 624 human JE cases with 19 deaths were reported in China.

JEV can infect a variety of animals through its natural replication cycle. Pigs act as an amplifying host and have a key role in JEV transmission in China; therefore, human infections are closely associated with the prevalence of JEV in pigs. To evaluate the potential risk of JEV infections among swine on human health, we examined all of the available data on JEV infections in China and investigated the dataset's geospatial epidemiologic features.

The JEV epidemiological data from 2006 to 2012 for swine herds in China were collected from the China National Knowledge Infrastructure database. A total of 22,343 serum samples were collected from pigs in 18 provinces and autonomous regions of China, including Guangxi, Guangdong, Zhejiang, Hunan, Jiangsu, Guizhou, Yunnan, Sichuan, Chongqing, Tibet, Hubei, Henan, Gansu, Hebei, Inner Mongolia, Liaoning, Jilin, and Heilongjiang (panel A in Fig. 1). Among those

samples, 8,807 (39.4%) were JEV-positive, and prevalence of JEV-positive samples varied by year (range, 23.0%–59.2%); the highest (59.2%, 3,286/5,548) occurring in 2010 and the lowest (23.0%, 417/1,810) in 2007 (panel B in Fig. 1). The distribution of JEV-positive cases also varied among the assessed geographic regions (range, 9.6%–60.6%) with the highest (60.6%, 1,575/2,597) in Guangxi province and the lowest (9.6%, 35/365) in Hebei province (panel A in Fig. 1). Geographically, JEV was more prevalent in southern China; the JEV-positive rate gradually decreased from south to north, similar to the drop in average daily temperature along the same path.

Numerous analyses have indicated that the incidence of JEV infection is seasonal and closely related to geographical distribution and climate [2]. Generally, peaks in human JEV epidemics in China occur for 7–9 months each year, which varies geographically from 5–6 months in southernmost Hainan province, to 6–7 months in southern China, to 8–9 months in northern China (panel C in Fig. 1), further indicating that the prevalence of JEV infections coincides with changes in average temperatures and the seasonal distribution of mosquitoes. Analyses of JEV serological data from Chinese swine herds showed that the prevalence and distribution of JEV in swine

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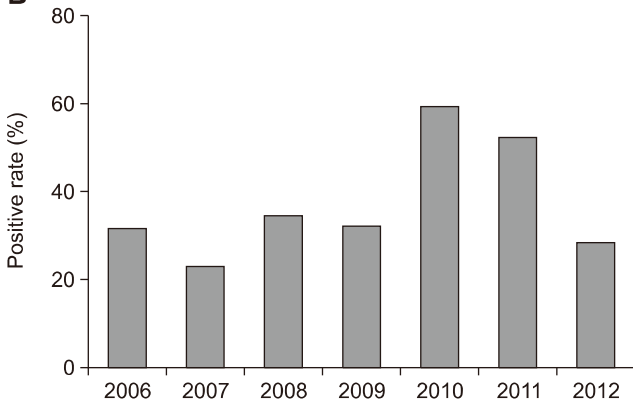
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A



B



C

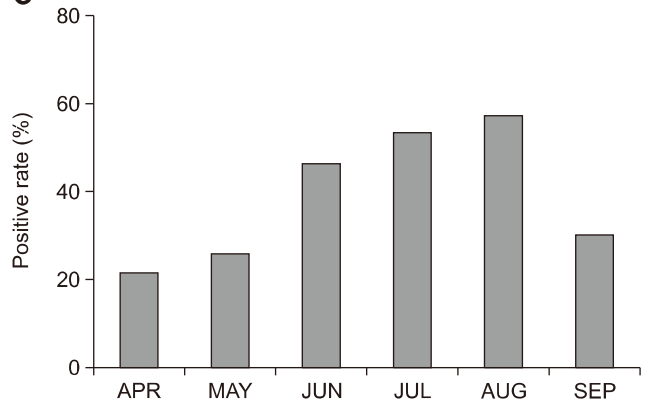


Fig. 1. The prevalence and distribution of Japanese encephalitis virus (JEV) in swine herds in China, 2006 to 2012. Serum samples were collected from swine herds in China, thereby providing JEV epidemiological data for different regions and provinces (A), years (B), and months (C).

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	KF297916	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	KF297915	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	<i>Homo sapiens</i> 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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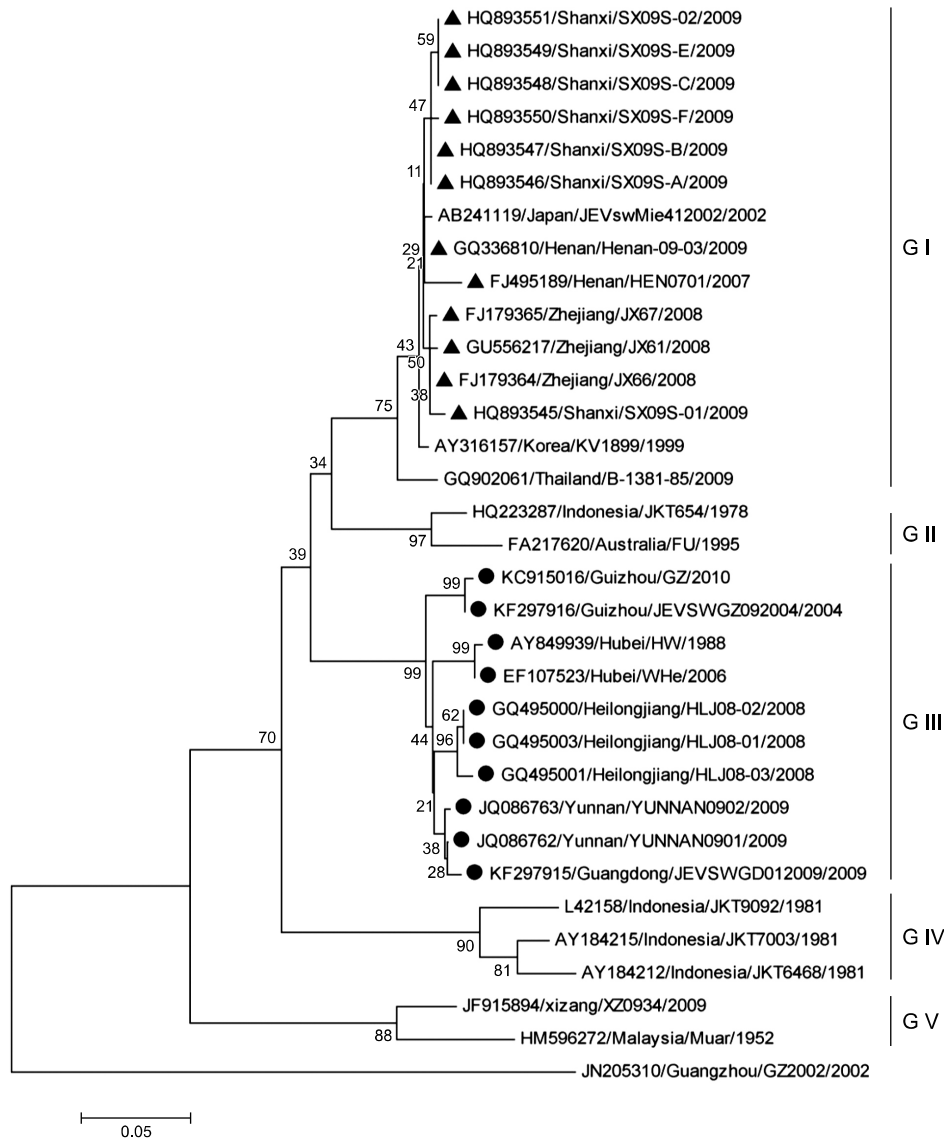


Fig. 2. Results of phylogenetic analysis of Japanese encephalitis virus strains isolated from Chinese swine herds based on assessment of the capsid/premembrane protein (C/PrM) nucleotide sequences. The multiple sequence alignments were obtained by using MEGA software (ver. 5.0; Molecular Evolutionary 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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