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販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		Yu H, Zhou YJ, Li GX, Zhang GH, Liu HL, Yan LP, Liao M, Tong GZ. Virus Res. 2009 Mar;140(1-2):85-90. Epub 2009 Jan 3.	中国

研究報告の概要

○中国においてブタがヒト様H1N1インフルエンザウイルスに感染しているさらなるエビデンス
典型的ブタおよびトリ様H1N1インフルエンザウイルスは世界中のブタから数多く報告されているが、ヒト様H1N1ブタ・ウイルスの報告は少ない。2006年にヒト様H1N1ブタ・ウイルス(A/swine/Guangdong/96/06)が広東省のブタから分離されたが、これは中国で初めての報告であった。ブタにおけるヒト様H1N1インフルエンザウイルス感染の更なる証拠を得るため、中国で分離された3つのヒト様ブタH1N1ウイルス(A/swine/Guangdong/96/06、A/swine/Tianjin/01/04、A/swine/Henan/01/06)の8つの遺伝子セグメントを分析した。3ウイルスにおける8つの遺伝子セグメントは、いずれも、最近(2000年頃)および早期(1980年代)のヒトH1N1インフルエンザウイルスと高い相同性を示した。系統発生解析では、A/Swine/Guangdong/96/06は、2000年頃のヒトH1N1インフルエンザウイルスに直接由来し、他のウイルス2種は、1980年代に循環したヒトH1N1ウイルスに由来すると考えられることが判明した。我々の分離株(A/swine/Guangdong/96/06)の血清陽性率から、中国のブタにヒト様H1N1ウイルスが存在することが確認された。これらインフルエンザウイルス(特に過去のウイルスであるA/swine/Tianjin/01/04とA/swine/Henan/01/06)の存在は、ヒト様H1N1インフルエンザウイルスがブタにおいて長期間不変でいることを示しており、ブタがヒト・パンデミックを引き起こす古いインフルエンザウイルスの保有宿主である証拠を示している。

報告企業の意見	今後の対応
中国のブタからヒト様H1N1インフルエンザウイルスが検出され、ブタがヒト・パンデミックを引き起こす古いインフルエンザウイルスの保有宿主である証拠が示されたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で発熱などの体調不良者を献血不適としている。更に、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該製剤の回収と医療機関への情報提供を行うこととしている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Further evidence for infection of pigs with human-like H1N1 influenza viruses in China

Hai Yu[a], Yan-Jun Zhou[a], Guo-Xin Li[a], Gui-Hong Zhang[b], Hui-Li Liu[c], Li-Ping Yan[a], Ming Liao[b], Guang-Zhi Tong[a,*]

[a] Division of Swine Infectious Diseases, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China
[b] College of Veterinary Medicine, Southern China Agricultural University, Guangzhou 510642, China
[c] Institute of Animal Sciences and Veterinary Medicine, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China

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ABSTRACT

Classical swine and avian-like H1N1 influenza viruses were reported widely in swine population worldwide, but human-like H1N1 swine viruses were reported occasionally. In 2006, a human-like H1N1 swine virus (A/swine/Guangdong/96/06) was isolated from pigs in Guangdong province, which was reported in China for the first time. To get further evidence for infection of pigs with human-like H1N1 influenza viruses, we analyzed eight gene segments of three human-like swine H1N1 viruses (A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06) isolated in China. All the eight genes of the three viruses are highly homologous to recent (about 2000) and early (1980s) human H1N1 influenza viruses, respectively. Phylogenetic analyses revealed that A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses, while A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s. Seroprevalence of our isolate (A/swine/Guangdong/96/06) confirmed the presence of human-like H1N1 virus in pigs in China. Existence of these influenza viruses, especially older viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06), indicates that human-like H1N1 influenza viruses may remain invariant for long periods in pigs and provides the evidence that pigs serve as reservoirs of older influenza viruses for human pandemics.

1. Introduction

Swine influenza is an acute respiratory disease caused by influenza A virus within the Orthomyxoviridae family. The primary clinical manifestations of viral infection are fever and acute respiratory distress. Currently, three main subtypes of influenza viruses are circulating in the swine population throughout the world: subtypes H1N1, H3N2 and H1N2 (Brown, 2000). These include classical swine H1N1, avian-like H1N1, human-like or avian-like H3N2, reassortant H3N2 and various genotype H1N2 viruses (Brown, 2000; Qi and Lu, 2006; Webby et al., 2000). These viruses have remained largely endemic in pig populations worldwide and have been responsible for one of most prevalent respiratory diseases in pigs.

China, especially southern China, is regarded as an epicenter of pandemic influenza viruses throughout history (Shortridge and Stuart-Harris, 1982). The tracheal epithelium in pigs expresses receptors for both human and avian influenza viruses, and this provides a biological basis for the susceptibility of pigs to both avian and human influenza viruses (Ito et al., 1998; Peiris et al., 2001). Pi can therefore function as intermediate hosts or "mixing vessels" in establishing new influenza virus lineages by supporting coinfection, replication, and reassortment among human, avian, and swine influenza viruses (Brown, 2000; Landolt et al., 2003). In the past, a number of influenza viruses have been isolated from pigs in China. These mainly include classical swine H1N1 viruses, avian-like H1N1 viruses, human-like H3N2 viruses, double-reassortant H3N2 viruses containing genes from the human and avian influenza viruses, triple-reassortant H3N2 viruses containing genes from the human, classical swine and avian viruses, avian-like H9N2 viruses, and double-reassortant H1N2 virus containing genes similar to those of human and swine viruses (Guan et al., 1996; Peiris et al., 2001; Shortridge and Webster, 1979; Xu et al., 2004; Yu et al., 2008a,b).

Human H1N1 viruses can infect pigs and pig-to-pig transmission has been demonstrated under experimental conditions (Brown, 2000). Serological surveillance studies worldwide suggest that the prevailing human H1N1 strains are readily transmitted to pigs and

* Corresponding author at: Division of Swine Infectious Diseases, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518, Ziyue Road, Minhang District, Shanghai 200241, China. Tel.: +86 21 34293436; fax: +86 21 54081818.
E-mail address: gztong@shvri.ac.cn (G.-Z. Tong).

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have resulted occasionally in the isolation of virus (Katsuda et al., 1995; Nerome et al., 1982; Yu et al., 2007). In 2006, a human-like H1N1 swine virus (A/swine/Guangdong/96/06) was isolated from pigs in Guangdong province, which was reported by us in China for the first time (Yu et al., 2007). To get further evidence for infection of pigs with human-like H1N1 influenza viruses, we made full use of our isolate and another two human-like H1N1 swine influenza viruses isolated and sequenced by scientists of Huazhong Agricultural University of China, and we analyzed their genetic evolution. In this study, we summarize and report, for the fist time, the coexistence of recent (about 2000) human-like and early (1980s) human-like swine H1N1 influenza viruses in pigs in China.

2. Materials and methods

2.1. Viruses

A/swine/Guangdong/96/06(H1N1) was isolated from pigs in a farm of Guangdong province of southern China, by inoculation into and subsequent passage in the allantoic cavity of 10-day-old SPF embryonated chicken eggs (Yu et al., 2007). Viral gene sequencing was carried out as follows. In brief, viral RNA was directly extracted from infected allantoic fluids using RNeasy Mini Kit (Qiagen, Chatsworth, CA) and reverse transcription (RT) were carried out under standard conditions using Uni12 (AGCAAAAGCAGG) primer. PCR was performed using specific primers for eight genes (primer sequences are available on request). PCR products were purified with the QIA quick PCR purification Kit (Qiagen, Inc.) and cloned into pMD18-T vector (TaKaRa, Dalian), then sequenced using synthetic oligonucleotides by Invitrogen Company.

In addition, A/swine/Tianjin/01/04(H1N1) and A/swine/Henan/01/06(H1N1) were isolated and sequenced by scientists of Huazhong Agricultural University of China. The nucleotide sequences were made available in GenBank under accession numbers: EU004440-EU004455.

2.2. Serum samples of pigs

From 2006 to 2007, we carried out swine influenza virus surveillance in China, a total of a total of 717 serum samples were randomly collected from apparently healthy pigs from nine provinces (Heilongjiang, Henan, Shandong, Zhejiang, Anhui, Jiangxi, Guangdong, Guangxi and Beijing).

2.3. Sequence analysis

All eight-gene segments of these three H1N1 swine influenza viruses were characterized and phylogenetically together with the representative sequence data available in GenBank. Sequence data were compiled and edited by using the Lasergene sequence analysis software package (DNASTAR Inc., Madison, WI). Multiple sequence alignment was carried out by using CLUSTAL W, and the unrooted phylogenetic trees were generated by the distance-based neighbor-joining method using MEGA 3.1. Bootstrap values were calculated on 1000 replicates of the alignment.

2.4. Serology tests

All sera were pretreated with the "Trypsin-Heat-Periodate" method to abolish interference by nonspecific serum inhibitors and used for hemagglutination inhibition (HAI) tests using chicken erythrocytes (World Health Organization, 2002). Neutralization tests were carried out by mixing 100 50% tissue culture infective doses of the virus with serial dilutions of serum and incubating for 2 h followed by inoculation onto MDCK cells grown in 96-well microtiter plates. After adsorption of the virus–serum mixture for 2 h, the inoculum was removed and fresh serum-free tissue culture medium containing trypsin (2 μg/ml) was added. Complete neutralization of cytopathic effect (read under an inverted microscope) was considered evidence of neutralizing antibody (Peiris et al., 2001; World Health Organization, 2002).

3. Results

3.1. Homology analysis of nucleotide sequences

Analysis of the homology of nucleotide sequences of eight genes of our isolate (A/swine/Guangdong/96/06) and another two isolates (A/swine/Tianjin/01/04 and A/swine/Henan/01/06) was performed by comparison with sequences available in GenBank (Table 1). All eight-gene segments of A/swine/Guangdong/96/06 were similar to H1N1 influenza viruses circulating in human in 2000 or 2001, with homologies ranging from 98.8 to 99.6%. But interestingly, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 were closely related to human H1N1 viruses isolated in 1980s, with homologies ranging from 98.2 to 100%.

3.2. Phylogenetic relationship of H1N1 swine influenza viruses from China

In the swine influenza virus surveillance in eight provinces (Heilongjiang, Henan, Shandong, Guangdong, Zhejiang, Anhui, Jiangxi, and Beijing) during 2005–2006, one human-like H1N1 influenza virus (A/swine/Guangdong/96/06) was isolated from pigs, which was reported in China for the first time (Yu et al., 2007). Recently, the sequences of two human-like H1N1 swine viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06) were published in GenBank. To characterize the gene segments of the three human-like H1N1 influenza viruses from pigs more precisely, we constructed the phylogenetic trees using the nucleotide sequences of the HA, NA, PB1, PB2, PA, NP, M and NS genes available in GenBank and the information from the trees was analyzed.

Phylogenetic analysis of the HA gene reveals that all of the H1N1 swine viruses isolated in China can be separated into three lineages, including human strains, classical swine strains and avian strains (Fig. 1). Previously most of the H1N1 swine influenza viruses, isolated in China, belong to classical swine or avian lineage. Classical swine lineage mainly includes A/swine/Guangdong/711/01, A/swine/Hong Kong/273/94, A/swine/Beiing/47/91, A/swine/Hong Kong/172/93 and so on. A/swine/Hong Kong/168/93 and A/swine/Hong Kong/176/93, had emerged in China, belong to avian lineage. A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 are incorporated into the human lineage. Our isolate (A/swine/Guangdong/96/06) was closely related to A/Dunedin/2/00, while A/swine/Tianjin/01/04 and A/swine/Henan/01/06 were derived from A/Memphis/12/86.

Phylogenetic analyses of NA, PB1, PB2, PA (Fig. 2), NP, M and NS (data not shown) genes showed a clear division of each of these genes into different lineages including classical swine lineage, human lineage and avian lineage, similar to the HA gene. A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 belong to human lineage in the seven phylogenetic trees. Because of the lack of sequence data of swine H1N1 influenza viruses isolated in China, these genes of classical swine lineage and avian lineage of China were not analyzed.

Based on the phylogenetic trees and homology of the nucleotide sequence of gene segments of the three viruses, A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses. But A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s.

Table 1
Genetic homology of the human-like swine influenza viruses isolated in China with related sequences available in GenBank.

Viruses	Gene	Virus with the highest identity	Identity (%)	GenBank accession no.
A/swine/Guangdong/96/06	HA	A/Dunedin/2/00(H1N1)	99.6	CY011584
	NA	A/Canterbury/43/00(H1N1)	99.4	CY010094
	PB1	A/New York/233/00(H1N1)	99.2	CY002646
	PB2	A/New York/443/01(H1N1)	99.4	CY003479
	PA	A/New York/443/01(H1N1)	99.1	CY003477
	NP	A/New York/234/00(H1N1)	99.3	CY002651
	M	A/New York/443/01(H1N1)	98.8	CY003473
	NS	A/New York/443/01(H1N1)	99.0	CY003476
A/swine/Tianjin/01/04	HA	A/Suita/1/89(H1N1)	99.0	D13573
	NA	A/Yamagata/120/86(H1N1)	99.1	D31948
	PB1	A/Singapore/6/86(H1N1)	99.8	CY020483
	PB2	A/New York/2924-1/86(H1N1)	99.6	CY021740
	PA	A/Fiji/15899/83(H1N1)	100.0	AJ605762
	NP	A/New York/2924-1/86(H1N1)	99.2	CY021736
	M	A/Singapore/6/86(H1N1)	98.4	CY020478
	NS	A/Chile/1/83/(H1N1)	98.2	X15282
A/swine/Henan/01/06	HA	A/Suita/1/89(H1N1)	98.9	D13573
	NA	A/Singapore/6/86(H1N1)	99.6	CY020479
	PB1	A/Singapore/6/86(H1N1)	99.9	CY020483
	PB2	A/New York/2924-1/86(H1N1)	99.3	CY021740
	PA	A/New York/2924-1/86(H1N1)	99.5	CY021738
	NP	A/New York/2924-1/86(H1N1)	99.2	CY021736
	M	A/Singapore/6/86(H1N1)	99.8	CY020478
	NS	A/Chile/1/83/(H1N1)	98.3	X15282

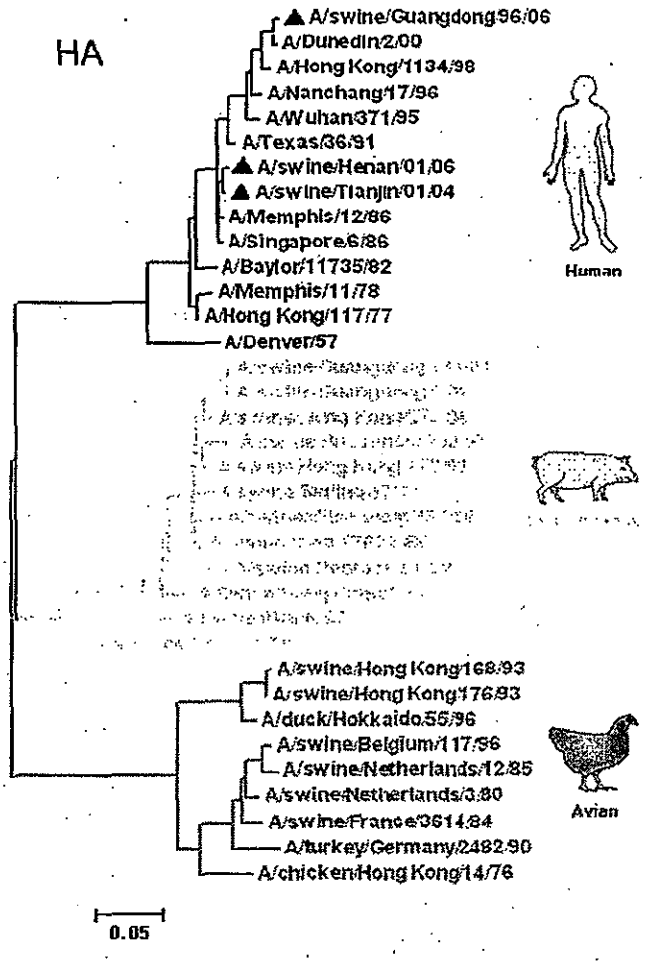

Fig. 1. Phylogenetic tree of the HA (positions 84–1061) gene of the H1N1 influenza viruses. The unrooted phylogenetic tree was generated by the distance-based neighbor-joining method using MEGA 3.1. Reliability of the tree was assessed by bootstrap analysis with 1000 replications, only bootstraps values >90% were shown. Different lineages are marked with different colors.

3.3. Molecular analysis

To try to identify possible determinants of interspecies transmission of H1N1 influenza viruses from human to pigs, the deduced amino acid sequences of HA1 region were aligned. The proposed antigenic sites (Caton et al., 1982; Lubeck and Gerhard, 1981; Olsen et al., 1993), receptor-binding sites (Nobusawa et al., 1991) and potential glycosylation sites were analyzed (Fig. 3).

Antigenic sites are regions of molecules involved in antibody binding and four sites (Sa, Sb, Ca and Cb) of H1N1 influenza virus have been defined (Caton et al., 1982; Wiley et al., 1981). A/swine/Guangdong/96/06 and A/Dunedin/2/00 have the same amino acids in antigenic sites, while A/swine/Tianjin/01/04, A/swine/Henan/01/06 and A/Memphis/12/86 also have the same amino acids in antigenic sites, which indicate these three viruses may have the similar antigenicity to rece (about 2000) and early (1980s) human H1N1 influenza virus respectively.

The host range of influenza A viruses is associated with differences in specificity of HA for attachment to sialic acid-containing receptors on susceptible cells. So the receptor-binding property of the HA protein of influenza virus is an important molecular determent of host-range restrictions (Matrosovich et al., 2000; Weis et al., 1988). The amino acids at positions 91, 131–135, 150, 180, 187, 191, 192, and 221–226 (98, 134–138, 153, 183, 190, 194, 195, and 224–229 according to H3 number) are components of receptor-binding sites of the HA of H1N1 influenza viruses (Nobusawa et al., 1991). The three human-like H1N1 swine influenza viruses and the two reference human viruses (A/Dunedin/2/00 and A/Memphis/12/86) had the same amino acids at Y^{91}, G^{131}, V^{132}, A^{134}, S^{135}, W^{150}, T^{152}, H^{180}, Y^{192}, R^{221}, Q^{223}, E^{224}, G^{225}, and R^{226} (receptor-binding sites). At position 133, the three swine influenza viruses and A/Dunedin/2/00 had the same amino acids (S). At position187, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 had the unique amino acid (E). The two amino acids of the three human-like swine influenza viruses at positions 191 and 222 were identical to A/Dunedin/2/00 and A/Memphis/12/86, respectively.

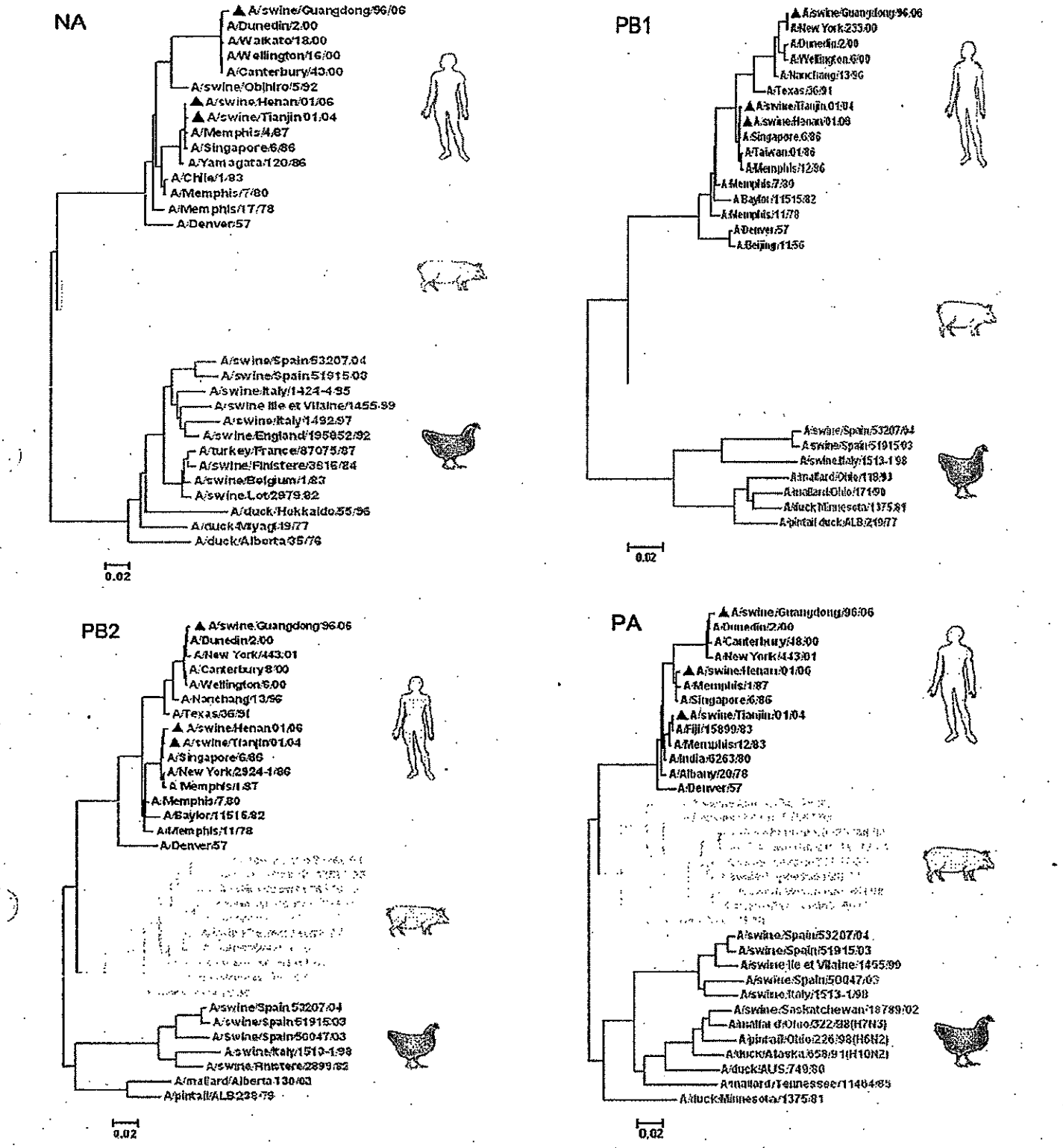

Fig. 2. Phylogenetic trees of the NA (positions 93–1415), PB1 (positions 14–2286), PB2 (positions 52–2295) and PA (positions 40–2175) genes of the H1N1 influenza viruses. The method used is as given in the legend of Fig. 1. Different lineages are marked with different colors.

Some glycosylation sites have a significant effect on receptor-binding property of the influenza virus HA protein, and glycosylation is therefore an important process in the generation of new virus (Schulze, 1997). Eight potential glycosylation sites (N-X-S/T) were conserved at positions 10, 11, 23, 54, 87, 125, 160, and 287 in the HA1 protein of the three human-like H1N1 swine influenza viruses and the two reference human viruses.

3.4. Seroprevalence of the human-like H1N1 influenza viruses in swine populations of China

The isolation and genetic characterization of human-like H1N1 influenza viruses in pigs suggested that these viruses might form a stable lineage in swine populations in China. So we conducted a serological surveillance to get some useful information about

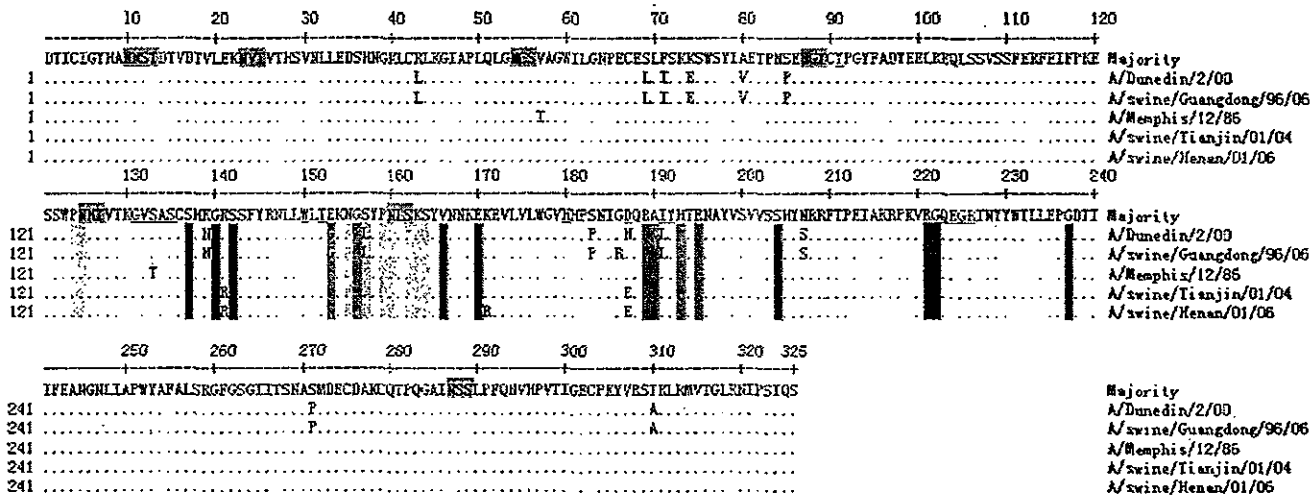

Fig. 3. Molecular analysis of HA1 amino acid sequences of the three H1N1 swine influenza viruses and reference strains. Potential glycosylation sites are marked with pink shade. Previously defined antigenic sites are indicated: site Sa (green shade), site Sb (red shade), site Ca (blue shade), site Cb (yellow shade). Underlined residues are receptor-binding sites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Seroprevalene of the human-like H1N1 influenza virus in swine populations of China.[a]

Province or city	Number of sera collected	HAI positive rate (%)	NT positive rate[b] (%)
Henan	68	17.6	11.8
Shandong	123	5.7	0
Heilongjiang	54	3.7	0
Zhejiang	92	7.6	6.5
Anhui	30	0	0
Jiangxi	44	4.5	2.3
Beijing	38	7.9	5.3
Guangxi	110	9.1	6.4
Guangdong	158	20.8	13.9

[a] HAI and neutralization positives were taken as titers of 1/80 or more.
[b] NT, neutralization test.

seroprevalence of the human-like H1N1 influenza viruses in swine populations of China. A collection of 717 pig serum samples from nine provinces in China was analyzed in HAI and neutralization tests for the presence of antibody to human-like H1N1 swine influenza virus (A/swine/Guangdong/96/06) (Table 2). Serological surveillance results indicated that the human-like H1N1 swine influenza virus might sporadically infect pigs in China. In the HAI test antibody to A/swine/Guangdong/96/06 was detected with prevalence ranging from 0 to 20.8%, while in the neutralizing test antibody to the H1N1 virus was relatively low with prevalence ranging from 0 to 13.9%.

4. Discussion

Influenza virus infection is an important cause of respiratory disease among pigs throughout the swine producing regions of the world (Karasin et al., 2000). Swine influenza was first observed in 1918 at the time of the human pandemic and the virus was isolated and identified in 1930 by shope (Brown, 2000; Shope, 1931). This virus was the prototype strain of a group of viruses now known as classical swine influenza viruses. Virologic and serological surveillance has shown that classical swine H1N1 is prevalent throughout the major pig population of the world (Brown, 2000; Chambers et al., 1991; Guan et al., 1996; Hinshaw et al., 1978). Since 1979, classical swine influenza viruses have been replaced by avian-like H1N1 viruses that are antigenically distinguishable from classical swine H1N1 viruses in Europe. Human H1N1 viruses can infect pigs and pig-to-pig transmission has been demonstrated under experimental conditions. Serological surveillance studies worldwide suggest that the prevailing human H1N1 strains are readily transmitted to pigs (Brown, 2000), but there are a few reports about isolation of the human-like swine H1N1 viruses. In China, classical swine H1N1 viruses were the predominant influenza virus infecting pigs and circulated in pigs in China in northern, central (Henan and Jiangxi), and southern (Guizhou and Guangdong) provinces (Guo et al., 1992). Since 1993, avian-like swine influenza viruses had been isolated from pigs and circulated with classical H1N1 viruses (Guan et al., 1996). In 2006, human-like swine H1N1 influenza viruses were reported by us for the first time. In this study, we summarized and reported coexistence of recent (about 2000) and early (1980s) human-like swine H1N1 influenza viruses, which provides further evidence for infection of pigs with human-like H1N1 influenza viruses in China.

Serological surveillance had indicated that classical swine H1 and human-like H3 subtype influenza infections widely existed in the pig populations in China, and avian H4, H5 and H9 influenza viruses had been transmitted to pig populations in southeastern China (Li et al., 2004; Ninomiya et al., 2002). No type of swine influenza vaccine has been used in pigs in China, and therefore the serological surveillance of human-like H1N1 swine influenza viruses conducted in this study could reflected the real situation of swine influenza infection. In this study, a total of 717 pig serum samples from nine provinces in China were detected in HAI and neutralization tests for the presence of antibody to human-like H1N1 swine influenza virus (A/swine/Guangdong/96/06). In the HAI test antibody to A/swine/Guangdong/96/06 was detected with prevalence ranging from 0 to 20.8%, while in the neutralizing test antibody to the H1N1 virus as relatively low with prevalence ranging from 0 to 13.9%. All these indicated that the human-like H1N1 swine influenza virus might sporadically infect pigs in China.

Influenza virus genomes are well known to undergo antigenic drift or antigenic shift that enable escape from preexisting immunity and cause new outbreaks of influenza in animals and even humans (Chi et al., 2005; Potter, 2001; Subbarai and Joseph, 2007), so influenza viruses exhibit the greatest genetic diversity and change every year. In this study, we analyzed eight gene segments of three human-like swine H1N1 viruses (A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06) isolated in China. Why were all the eight genes of the three viruses closely related to recent (about 2000) or early (1980s) human H1N1 influenza viruses? A possible explanation may be that these influenza viruses were introduced into

pigs at the time they circulated in humans and have persisted in pigs without antigenic drift. In China, Pigs have a short lifespan (approximately 6 months) and are not inoculated any type of swine influenza vaccine. Once the influenza viruses were introduced into pigs, these viruses might appear to have been under less immune selection pressure and all genes evolved more slowly than in humans and poultry. We describe here genetic relatedness of these swine isolates with recent (about 2000) or early (1980s) human H1N1 influenza viruses and provide evidence of long term conservation of human H1N1 influenza viruses in pigs.

Of the four pandemic strains of human influenza A virus occurred in the 20th century, the 1977 pandemic strain was very similar in all eight genes to a 1950 human H1N1 strain (Kilbourne, 2006). Therefore, pandemic strains of influenza A virus could arise by re-emergence of these older viruses that may have caused an epidemic many years earlier. In this study, we phylogenetically analyzed eight gene segments of three human-like H1N1 influenza viruses isolated from pigs in China. A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses. But A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s, which owed the possibility that pigs serve as reservoirs for older influenza viruses.

China, especially Southern China, is thought to be the epicenter for the human influenza pandemics throughout history (Shortridge and Stuart-Harris, 1982). The special environment and lifestyle in southern China provide more chances for wild aquatic birds, domestic poultry, pigs and humans to contact closely, and create the opportunity for interspecies transmission and generation of new reassortment influenza viruses. Although, it is virtually impossible to prevent new outbreaks of influenza in human and animals, it is now well recognized that animal influenza virus surveillance can play a key role in the early recognition of outbreak threats. So it is of great significance to carry out swine influenza virus surveillance. Existence of these influenza viruses, especially older viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06), in pigs provides the evidence that pigs serve as reservoirs of older influenza viruses for human pandemics and emphasizes the importance of reinforcing swine influenza virus surveillance in China.

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