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Short communication

Genetic diversity of H9N2 influenza viruses from pigs in China: A potential threat to human health?

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A B S T R A C T

Pandemic strains of influenza A virus might arise by genetic reassortment between viruses from different hosts. Pigs are susceptible to both human and avian influenza viruses and have been proposed to be intermediate hosts or mixing vessels, for the generation of pandemic influenza viruses through reassortment or adaptation to the mammalian host. In this study, we summarize and report for the first time the coexistence of 10 (A–J) genotypes in pigs in China by analyzing the eight genes of 28 swine H9N2 viruses isolated in China from 1998 to 2007. Swine H9N2 viruses in genotype A and B were completely derived from Y280-like and Shanghai/F/98-like viruses, respectively, which indicated avian-to-pig interspecies transmission of H9N2 viruses did exist in China. The other eight genotype (C–J) viruses might be double-reassortant viruses, in which six genotype (E–J) viruses possessed 1–4 H5-like gene segments indicating they were reassortants of H9 and H5 viruses. In conclusion, genetic diversity of H9N2 influenza viruses from pigs in China provides further evidence that avian to pig interspecies transmission of H9N2 viruses did occur and might result in the generation of new reassortant viruses by genetic reassortment with swine H1N1, H1N2 and H3N2 influenza viruses, therefore, these swine H9N2 influenza viruses might be a potential threat to human health and continuing to carry out swine influenza virus surveillance in China is of great significance.

Swine influenza is an acute respiratory disease caused by influenza A virus within the Orthomyxoviridae family. Infection of pigs with influenza A viruses is of substantial importance to the swine industry and to the epidemiology of human influenza (Landolt et al., 2003). At present, three main subtypes of influenza viruses are circulating in the swine population worldwide: 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 the most prevalent respiratory diseases in pigs. In China, many influenza viruses have been isolated from pigs. These mainly include classical swine H1N1 viruses, avian-like H1N1 viruses, double-reassortant H1N2 viruses containing genes similar to those of human and swine 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 and avian-like H9N2 viruses (Guan et al., 1996; Peiris et al., 2001; Shortridge and Webster, 1979; Xu et al., 2004; Yu et al., 2007, 2008a,b, 2009b)

Pandemic strains of influenza A virus might arise by genetic reassortment between viruses from different hosts. The human influenza pandemic strains of 1957 (Asian/

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57(H2N2)) and 1968 (Hong Kong/68(H3N2)) were the result of reassortment, which were double-reassortant viruses containing genes similar to those of avian and human influenza viruses (Kawaoka et al., 1989; Scholtissek et al., 1978). The reassortment events responsible for 1957 and 1968 pandemic viruses might occur in an animal that served as a "mixing vessel." Pigs are susceptible to both human and avian influenza viruses and have been proposed to be intermediate hosts for the generation of pandemic influenza viruses through reassortment (Fouchier et al., 2003; Ito et al., 1998; Scholtissek et al., 1993). So, the human influenza pandemic strains of 1957 and 1968 might have been generated in pigs (Fouchier et al., 2003). In the 2009 influenza pandemic, a novel swine-origin influenza A (H1N1) virus has attracted global attention and brought an overwhelming fear, which indicated the genetic reassortment of swine, avian and human influenza viruses is great threat to human health (Dawood et al., 2009).

The H9N2 subtype virus is a notable member of the influenza family because it can infect not only chickens, ducks and pigs, but also humans (Butt et al., 2005; Cong et al., 2007; Peiris et al., 1999, 2001; Shi et al., 2008; Xu et al., 2004, 2007). In China, the H9N2 virus was first isolated from a chicken in Guangdong province in 1992 (Chen et al., 1994; Cong et al., 2007) and now is the most prevalent subtype of influenza virus in poultry in China (Li et al., 2005). Recent studies have demonstrated that swine H9N2 virus can infect pigs and cause significant morbidity and mortality (Cong et al., 2007; Xu et al., 2004). To learn the overall information about the prevalence of swine H9N2 influenza and provide useful data for swine influenza control, and possibly also some necessary information for the prediction and preparedness of future human influenza pandemics, we make full use of sequences available in GenBank from 1998 to 2005 and sequences of our swine H9N2 viruses isolated in 2007 (Yu et al., 2008a), and analyze their genetic evolution. In the study, for the first time, we summarize and report the coexistence of ten genotypes of swine H9N2 influenza viruses in pigs in China.

From 2005 to 2007, we carried out swine influenza virus surveillance in China. A total of 650 samples including nasal swabs, lungs, and trachea were collected from pigs showing clinical symptoms (coughing, sneezing, nasal discharge, difficult breathing and depressed appetite) in 10 provinces, including Heilongjiang, Henan, Shandong, Guangdong, Zhejiang, Anhui, Jiangxi, Guangxi, Beijing, and Shanghai. Eleven influenza A viruses were isolated using 10-day-old SPF chicken eggs and identified by hemagglutination inhibition (HI) test and neuraminidase inhibition (NI) test using a panel of reference sera (National Reference Laboratory for Avian Influenza, Harbin Veterinary Research Institute, CAAS, China), including two H1N1 viruses, one H1N2 virus, four H3N2 viruses and four H9N2 viruses (Yu et al., 2007, 2008a, 2009a). Among these viruses, the four H9N2 viruses were named as follows: A/swine/Guangxi/7/07, A/swine/Guangxi/8/07, A/swine/Guangxi/9/07 and A/swine/Guangxi/10/07.

Viral gene sequencing was carried out as follows (Yu et al., 2007). 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

Table 1

GenBank accession numbers of gene segments of swine H9N2 influenza viruses isolated in China from 1998 to 2007.

Isolate	HA	NA	PB2	PB1	PA	NP	M	NS
A/swine/Hong Kong/9/98	AF222810	AF222812	AF222816	AF222818	AF222820	AF222814	AF222822	AF222824
A/swine/Hong Kong/10/98	AF222811	AF222813	AF222817	AF222819	AF222821	AF222815	AF222823	AF222825
A/swine/Shandong/na/03	DQ997419	DQ997421	DQ997418	DQ997417	DQ997424	DQ997422	DQ997420	DQ997423
A/swine/Shandong/nb/03	DQ997428	DQ997429	DQ997427	DQ997426	DQ997432	DQ997430	DQ997425	DQ997431
A/swine/Shandong/fHZ/03	DQ981602	DQ981604	DQ981599	DQ981600	DQ981601	DQ981603	DQ981605	DQ981606
A/swine/Shandong/fNY/03	DQ981610	DQ981612	DQ981607	DQ981608	DQ981609	DQ981611	DQ981613	DQ981614
A/swine/Shandong/fLS/03	DQ981618	DQ981620	DQ981615	DQ981616	DQ981617	DQ981619	DQ981621	DQ981622
A/swine/Shandong/fZC/03	DQ981626	DQ981628	DQ981623	DQ981624	DQ981625	DQ981627	DQ981629	DQ981630
A/swine/Shandong/w4/03	EU516304	EU516305	EU516309	EU516310	EU516311	EU516308	EU516306	EU516307
A/swine/Shandong/fJN/03	DQ981578	DQ981580	DQ981575	DQ981576	DQ981577	DQ981579	DQ981571	DQ981582
A/swine/Henan/2/04	DQ981586	DQ981588	DQ981583	DQ981584	DQ981585	DQ981587	DQ981589	DQ981590
A/swine/Henan/3/04	DQ981538	DQ981540	DQ981535	DQ981536	DQ981537	DQ981539	DQ981541	DQ981542
A/swine/Henan/4/04	DQ981546	DQ981548	DQ981543	DQ981544	DQ981545	DQ981547	DQ981549	DQ981550
A/swine/Henan/5/04	DQ981562	DQ981564	DQ981559	DQ981560	DQ981561	DQ981563	DQ981565	DQ981566
A/swine/Henan/6/04	DQ981594	DQ981596	DQ981591	DQ981592	DQ981593	DQ981595	DQ981597	DQ981598
A/swine/Henan/7/04	DQ981554	DQ981556	DQ981551	DQ981552	DQ981553	DQ981555	DQ981557	DQ981558
A/swine/Henan/8/04	DQ981570	DQ981572	DQ981567	DQ981568	DQ981569	DQ981571	DQ981573	DQ981574
A/swine/Jiangxi/1/04	EU502893	EU502894	EU502898	EU502899	EU502900	EU502897	EU502895	EU502896
A/swine/Jiangxi/wx2/04	EU502901	EU502902	EU502906	EU502907	EU502908	EU502905	EU502903	EU502904
A/swine/Guangdong/wxl/04	EU516312	EU516313	EU516317	EU516318	EU516319	EU516316	EU516314	EU516315
A/swine/Guangxi/58/05	EF612742	EF612744	EF612749	EF612748	EF612747	EF612745	EF612743	EF612746
A/swine/Guangxi/FS2/05	EU086302	EU086306	EU086316	EU086314	EU086313	EU086309	EU086304	EU086310
A/swine/Guangxi/S15/05	EU086318	EU086322	EU086333	EU086331	EU086328	EU086324	EU086321	EU086326
A/swine/Guangxi/S11/05	EU086319	EU086323	EU086332	EU086330	EU086329	EU086325	EU086320	EU086327
A/swine/Guangxi/7/07	CY075030	CY075032	CY075027	CY075028	CY075029	CY075031	CY075033	CY075034
A/swine/Guangxi/8/07	CY075038	CY075040	CY075035	CY075036	CY075037	CY075039	CY075041	CY075042
A/swine/Guangxi/9/07	CY075046	CY075048	CY075043	CY075045	CY075045	CY075047	CY075049	CY075050
A/swine/Guangxi/10/07	CY075054	CY075056	CY075051	CY075052	CY075053	CY075055	CY075057	CY075058