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Research paper

Molecular evolution of H1N1 swine influenza in Guangdong, China, 2016–2017

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ABSTRACT

Swine are the main host of the H1N1 swine influenza virus (SIV), however, H1N1 can also infect humans and occasionally cause serious respiratory disease. To trace the evolution of the SIV in Guangdong, China, we performed an epidemic investigation during the period of 2016–2017. Nine H1N1 influenza viruses were isolated from swine nasal swabs. Antigenic analysis revealed that these viruses belonged to two distinct antigenic groups, represented by A/Swine/Guangdong/101/2016 and A/Swine/Guangdong/52/2017. Additionally, three genotypes, known as GD52/17-like, GD493/17-like and GD101/16-like, were identified by phylogenetic analysis. Importantly, the genotypes including a minimum of 4 pdm/09-origin internal genes have become prevalent in China in recent years. A total of 2966 swine serum samples were used to perform hemagglutination inhibition (HI) tests, and the results showed that the seroprevalence values of SW/GD/101/16 (32.2% in 2016, 32.1% in 2017) were significantly higher than the seroprevalence values of SW/GD/52/17 (18.0% in 2016, 16.7% in 2017). Our study showed that the three reassortant genotypes of H1N1 SIV currently circulating in China are stable, but H1N1pdm09 poses challenges to human health by the introduction of internal genes into these reassortant genotypes. Strengthening SIV surveillance is therefore critical for SIV control and minimizing its potential threat to public health.

1. Introduction

Influenza is one of the most significant pandemic diseases that threatens human health. Influenza A virus (IAV) has been characterized by antigenic drift and antigenic shift, which adds complexity to the risk profiles for avian and mammalian populations (Shih et al., 2007; Smith et al., 2009; Treanor, 2004). Pigs are considered to play an important role in the evolution of IAV, as they may facilitate novel reassortment events with IAVs from different species and can cross over to humans (Garten et al., 2009; Kawaoka et al., 1989). In China, classical swine (CS) H1N1 viruses were predominant before 1988 (Vijaykrishna et al., 2011), but the persistent introduction of Eurasian avian-like swine (EA) viruses and triple-reassortant swine (TRIG) viruses resulted in the rapid expansion of genetic diversity of swine influenza viruses visible in recent years (Lam et al., 2011; Smith et al., 2009). The complicated genetic diversity of SIVs could became a highly potential threat to public health, particularly due to emergence of the swine-origin 2009 H1N1 pandemic influenza virus (Liang et al., 2014; Yang et al., 2016).

The EA H1N1 virus was first reported in 1979 in Europe (Scholtissek et al., 1983). Since 2005, it has spread to pig herds and has become dominant in China (Vijaykrishna et al., 2011). Previous studies have shown that the earliest EA reassortants (Sw/HK/72/2007), which displayed two amino acid changes in HA antigenic sites (Vijaykrishna et al., 2011), were genetically and antigenically different from other isolates and gradually became a distinct clade (Liang et al., 2014; Yang et al., 2016). Later, it was verified that this branch of IAVs isolates was present in pig herds (Yang et al., 2016), furthermore, EA H1N1 viruses acquired different internal gene segments from viruses in other lineages, resulting in an extremely complex and constantly evolving landscape (Liang et al., 2014; Sun et al., 2016; Yang et al., 2016).

Understanding the epidemic situation and molecular evolution of IAVs, especially the novel clade, contributes to the need for an effective outbreak alert and prevention plan. In this study, we performed a comprehensive phylogenetic analysis of EA H1N1 viruses and investigated their prevalence using a serological survey.

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2. Materials and methods

2.1. Sample collection

A total of 1294 nasal swab and lung tissue samples were collected from pig at commercial farms and abattoirs in Guangdong province during the period of 2016–2017. Nasal swab samples were collected and placed in viral medium and lung tissue samples were collected from different parts of the lungs. These samples were stored at 4 °C during the transporting process within 24 h, and frozen at −80 °C for future use.

2.2. Virus isolation

Nasal swab and lung tissue samples were inoculated into 9–10 day-old specific-pathogen-free (SPF) embryonated hen eggs or Madin-Darby canine kidney cells (MDCK) incubated for virus isolation. After 48–72 h, the allantoic fluid of the inoculated eggs or supernatant of the infected cell cultures was collected and tested for the presence of hemagglutinin, and was further confirmed by RT-PCR.

2.3. RNA extraction, whole-genome amplification and sequencing

Viral RNA of positive isolated samples was extracted using TRIzol reagents (Invitrogen, USA as per the manufacturer's manual). Each gene segment was amplified by using Ex Taq DNA polymerase (Takara) with the segment-specific primers as described previously (Poon et al., 2010; Vijaykrishna et al., 2010). The PCR products were collected and cloned into the pMD18-T vector (TaKaRa) and sequenced (performed by Shanghai Life Technologies Biotechnology Co., Ltd.). The data were analyzed with the SEQMAN program (DNASTAR, Madison, WI, USA). Nucleotide sequences obtained in our study are available from GISAID under accession numbers EPI1149964 to EPI1150035.

2.4. Phylogenetic analysis

On August 8, 2017, all EA H1N1 sequences generated in this study were collated from GenBank and the Global Initiative on Sharing Avian Influenza Data (GISAID). Each gene segment dataset was aligned with MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh et al., 2002; Katoh and Standley, 2013). The regions of each gene segment dataset were as follows: HA, 33 to 1733; NA, 21 to 1430; PB2, 28 to 2307; PB1, 28 to 2307; PA, 28 to 2307; NP, 46 to 1542; M, 26 to 1007; and NS, 27 to 864.The phylogenetic relationships of each gene segment were inferred by using the maximum likelihood (ML) method with PhyML 3.0 under the GTR + I + Γ_4 model and bootstrapped with 1000 replicates (Guindon et al., 2010). The resulting trees were displayed using FigTree v1.4.2.

2.5. Production of anti-sera

A/Swine/Guangdong/SS1/2012 H1N1 (SW/GD/SS1/12), A/Swine/Guangdong/SS11/2016 H1N1 (SW/GD/SS11/16), A/Swine/Guangdong/101/2016 H1N1 (SW/GD/101/16), A/Swine/Guangdong/MS285/2017 H1N1 (SW/GD/MS285/17), A/Swine/Guangdong/MS315/2017 H1N1 (SW/GD/MS315/17) and A/Swine/Guangdong/52/2017 H1N1 (SW/GD/52/17) were selected to perform antigenic analysis. The allantoic fluid containing virus was inactivated by adding 0.2% (*v*/v) formalin and mixed with an equal volume of Freund's adjuvant to form an emulsion. Rabbits were immunized four times (within two-week intervals) with 1 ml of vaccine by hypodermic injection. The serum samples were collected before each immunization and two weeks after the last immunization.

2.6. Antigenic analysis

The serum samples of rabbits were treated with receptor-destroying enzyme (RDE; Denka Seiken Co. Ltd., Tokyo, Japan). Hemagglutination inhibition (HI) was performed according to World Health Organization guidelines (WHO, 2011).

2.7. Serological survey

From May 2016 to July 2017, a total of 2966 swine serum samples were collected throughout Guangdong, Jiangxi, Anhui, Guangxi, Hunan and Fujian Provinces. SW/GD/101/16 and SW/GD/52/17 were used. All samples were tested by hemagglutination inhibition (HI) in a similar manner to that of the rabbit anti-sera preparation. A serum sample was considered positive when the HI titers ≥ 1:80.

3. Results

3.1. Virus isolation

From March 2016 to July 2017, a total of 1294 nasal swab and lung tissue samples were collected from pigs at commercial farms and abattoirs in Guangdong Province, China. Nine H1N1 subtype SIVs were isolated and named as A/Swine/Guangdong/FNPB/2016, A/Swine/Guangdong/CRT13/2016, A/Swine/Guangdong/101/2016, A/Swine/Guangdong/SS11/2016, A/Swine/Guangdong/52/2017, A/Swine/Guangdong/MS285/2017, A/Swine/Guangdong/MS315/2017, A/Swine/Guangdong/MS493/2017 and A/Swine/Guangdong/SS12/2017. No other subtypes were isolated. The overall rate of recovered virus by isolation was 0.70%.

3.2. Phylogenetic analyses of H1N1 SIVs

To investigate the genesis of the virus, we analyzed each gene segment of EA H1N1 (n = 255). As shown in Fig. 1 and Supplementary Fig. S1a, the HA genes of EA H1N1 viruses can be divided into Group 1 and Group 2, as both groups belong to the EA H1N1 lineage. Group 1 harbored more sequences than Group 2 and was more widely distributed. Group 2, represented by Sw/HK/72/2007, comprised 48 viruses that were only isolated in Hong Kong, Guangdong and Guangxi in China. Similar to HA, the NA genes of these isolates can also be grouped into two groups (Supplementary Fig. S1b). Interestingly, most NA genes for each group are also accompanied by HA genes for each group. Phylogenetic analysis of the HA and NA genes revealed that 8 of 9isolated viruses belonged to Group 1, the exception was SW/GD/52/17. The nucleotide sequence of the HA gene and NA genes of the 9 viruses shared 91.7%–99.8% and 90.3%–98.9% identity respectively.

As shown in Supplementary Fig. S1c–g, all internal genes other than MP and PB2 can be grouped into the following major three lineages: TRIG, 2009/H1N1 and EA Group 1. MP can be grouped into two lineages: 2009/H1N1 and EA Group 1. The most complicated is PB2, which can be grouped into 4 lineages: TRIG, 2009/H1N1 EA Group 1 and EA Group 2. Except for MP and NS, the other internal genes (PB2, PB1, PA and NP) of these nine viruses belonged to the 2009/H1N1 lineage, sharing 96.9%–100%, 97.4%–99.3%, 96.0%–99.1% and 96.9%–99.1% nucleotide identity, respectively. The MP gene shared 92.9–99.6% nucleotide identity and belonged to 2009/H1N1 and EA Group 1. As shown in Fig. 2 and Supplementary Fig. S1h, the NS genes were observed to be of TRIG lineage, whit a shared identity of 97.3–100%.

The results indicate that since the introduction of TRIG viruses, the virus evolved into HK72/07-like in 2007. This genotype established reassortants that subsequently reassorted with a “pure” EA genotype (like A/SW/HK/NS1179/07) and pdm/09 H1N1 to generate multiple reassortants. From 2013 to 2014, three novel genotypes, GD52/17-like, GD493/17-like and GD101/16-like, emerged and gradually became