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Pathogenic characteristics of a novel triple-reasserted H1N2 swine influenza virus

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ABSTRACT

A novel triple reasserted H1N2 virus A/swine/Shanghai/1/2007 (SH07) was isolated from nasal swabs of weaned pig showing clinical symptoms of coughing and sneezing. To explore the virus characteristics, mice, chickens and pigs were selected for pathogenicity study. Pigs inoculated intranasally with 10^6 $TCID_{50}$ SH07 showed clinical symptoms with coughing and sneezing, but no death. The virus nuclear acid was detected in many tissues using real-time PCR, which was mainly distributed in respiratory system particularly in the lungs. The virus was low-pathogenic to chickens with 10^6 $TCID_{50}$ dose inoculation either via intramuscular or intranasal routes. However virus nuclear acid detection and virus isolation confirmed that the virus can also be found in nasal and rectum. When virus was inoculated into mice by intramuscular or intranasal routes we observed 100% and 80% lethality respectively. The third generation of samples passaged on MDCK cell were SIV positive in indirect immunofluorescence assay (IFA) using antiserum against H1N2 SIV. Furthermore, the lungs of mice showed obvious lesion with interstitial pneumonia. Data in our study suggest that SH07 is preferentially pathogenic to mammals rather than birds although it is a reasserting virus with the fragments from swine, human and avian origin.

1. Introduction

Swine influenza was first recognized as a disease in 1918 pandemic [1]. Although the 1918 A (H1N1) pandemic virus appeared in swine and human populations around the same time, people did not know whether infected swine transmitted the virus to humans, by reverse transmitted path, or infection occurred at the same time [2]. Swine infected A influenza is associated with respiratory illness and often causes sudden unexpected deaths in piglets. In farms employing traditional farming methods, swine influenza disease is a seasonal illness which peaks in the colder months [1]. Influenza usually appears in a herd resulting from the introduction of infected animals, either from movement between nabbing farms or mixing infected pigs with susceptible pigs [3].

The first A (H1N1) viruses isolated from swine in the United States during 1930 were known as classical swine influenza A (H1N1) viruses [4]. A (H3N2) viruses were first identified in swine in 1970 during an influenza surveillance study in Taiwan [5]. Since the initial introduction of human A (H1N1) and A (H3N2) viruses were transmitted into swine populations, multiple reasserteds with variant genetic compositions have reported [4]. The first A (H1N2) swine influenza virus was reported in Japan during 1980, which was a classical swine A (H1N1) virus obtained neuraminidase (NA) from human A (H3N2) viruses [6]. Till now, H1N2 Swine influenza viruses were isolated in many countries [7–12]. In China, three influenza H1N2 isolates from pig samples were reported in 2004 and many H1N2 reasserted from variant genetic origin were reported [4]. A novel reasserted strain A/swine/Shanghai/1/2007 (H1N2) (SH07) was isolated from the nasal swabs of weaned pig with clinical symptoms. SH07 was identified to be a triple reasserted strain in our previous study, with its HA, NP, M and NS segments from classical swine lineage, NA and PB1 from human-origin, and PB2 and PA from avian origin [13]. Few studies have been reported to discover biological characteristics of naturally occurring triple ressortant H1N2 virus. In this report, we characterized the pathogenicity of SH07 in swine, chicken and mice models. We plan to perform further in depth studies to increase our understanding on mechanism of virus transmission.

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Table 1
Primers used in this study.

Primer	Primer sequences (5′-3′)	length (bp)
NP-1-F	GAGAAAGTCGGAACCCAGGAAC	111
NP-1-R	CACAAGCAGGCAGGCAAGAC	

2. Materials and methods

2.1. Ethics statement

All animal studies were conducted under the guidance of Institutional Animal Care and Use Committee in Centers for Disease Control and Prevention (CDC) and the Laboratory Animal Care International-accredited facility.

Viruses. A/swine/Shanghai/1/2007 (H1N2) (SH07) was isolated in Shanghai, China in 2014 from nasal swab of one weaned pig showing clinical symptoms (coughing, sneezing, nasal discharge, difficult breathing and depressed appetite) and preserved by Shanghai Municipal Key Laboratory of Animal Genetics Engineering [13]. For virus cultivation, Madin-Darby canine kidney (MDCK) cells was grew in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.5 μg/mL TPCK-treated trypsin at 37 °C with 5% CO_2. Viral fluids were harvested and used for further animal experiments.

2.2. Virus titer assay in MDCK cells

Ten-fold serial dilutions of virus were inoculated on MDCK cells, cell supernants were discarded and replaced with the fresh DMEM containing 0.5 μg/mL TPCK-treated trypsin after 1 h. Five days later,

A

	Days of sample collection										
	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d	11d
Nasal swabs	+	+	+	+	+	+	+	+	+	-	-
Rectum swabs	-	+	+	+	+	+	+	+	-	-	-

B

	Days of sample collection					
Tissues	3d	5d	7d	10d	12d	14d
Brain	7.9^10^{-2}	**1.9^10^{1}**	1.6^10^{-1}	4.0^10^{-2}	4.0^10^{-2}	3.1^10^{-2}
Lung	5.3^10^{-1}	**4.5^10^{6}**	**7.4^10^{8}**	**8.2^10^{5}**	6.1^10^{-1}	1.4^10^{-1}
Trachea	3.0^10^{-2}	**2.7^10^{6}**	**4.2^10^{7}**	**2.8^10^{4}**	2.4^10^{-2}	4.8^10^{-1}0
Larynx	**6.3^10^{3}**	**3.7^10^{4}**	**2.6^10^{5}**	**5.2^10^{3}**	3.1^10^{-1}	2.6^10^{-1}
Lung lymp note	5.9^10^{-1}	**6.4^10^{3}**	**3.1^10^{4}**	2.0^10^{-1}	1.6^10^{-2}	4.1^10^{-2}
liver	1.8^10^{-2}	5.0^10^{-2}	6.5^10^{-2}	8.1^10^{-1}	2.9^10^{-2}	3.5^10^{-2}
kidney	1.5^10^{-1}	8.6^10^{-1}	8.4^10^{-2}	1.2^10^{-1}	4.0^10^{-2}	4.0^10^{-2}
heart	2.7^10^{-2}	7.2^10^{-1}	9.2^10^{-1}	1.9^10^{-1}	4.0^10^{-2}	4.0^10^{-2}
spleen	3.9^10^{-2}	**2.3^10^{1}**	**3.5^10^{2}**	3.5^10^{-2}	4.0^10^{-2}	4.0^10^{-2}

C

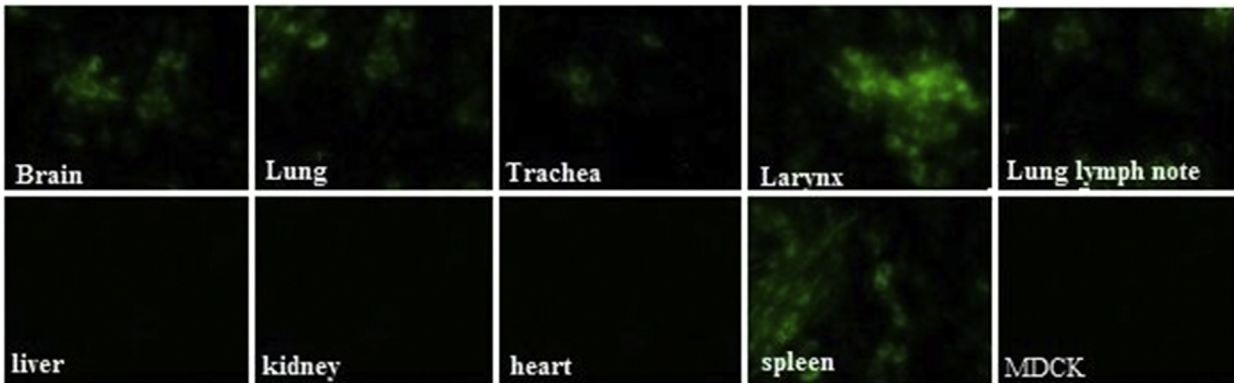

Fig. 1. Virus detection in tissue samples of infected pigs. (A) The nasal and rectum swabs of the pigs inoculated with SH07 and PBS were all collect and used for SH07 nucleic acid detection by real-time PCR. Ct values of the control samples were 39 and the RNA copies is 7.8 × 10^{-1} copies/μL This was used as the threshold value and the RNA copies which was more than it is considered to be positive and to be negative when less than the threshold value. (B) Brain, lung, trachea, larynx, lung lymph node, liver, kidney, heart and spleen samples of pigs were collected after inoculation and were used for virus detection by Real-time PCR. Data in the table were the nucleic acid content of the tissues according to the standard curve equation of Copies/ul = $10^{(-0.296\times Ct+10.150)}$. The liver, kidney and the heart were SIV negative during the whole experiment. The virus was mainly distributed in respiratory tracks, large amount of SIV SH07 RNA copies were detected in lung, trachea, larynx and lung lymph nodes. (C) Pig tissues of the brain, lung, trachea, larynx, lung lymph node and spleen collected at 5dpi were SIV positive after cultivation in MDCK cells by indirect immunofluorescence assay (IFA) using SIV antiserum against SH 07 virus. However the samples of liver, kidney and the heart were negative in IFA.