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Cloning and Phylogenetic Analysis of *NS1* Gene of H3N2 Subtype Swine Influenza Virus from Different Isolates in Chongqing China 2010-2011

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Abstract: Forty nine strains of H3N2 subtype swine influenza viruses identified by specific Reverse Transcription-PCR Method which were isolated from the swine in Chongqing, China during the 2010 to 2011. *NS1* genes of these strains were sequenced and analyzed with the representative strains published in GenBank. The results indicated that the *NS1* genes of these strains and the A/swine/Guang Dong/811/2006(H3N2) and Influenza A virus (A/swine/Colorado/1/1977(H3N2)), A/Netherlands/233/1982(H3N2), A/swine/Heilongjiang/1/05(H3N2) and A/swine/Fujian/F2/2007(H3N2) displayed nucleotide homologies nearly 99%. These results suggested that the strains might be derived from human influenza.

Key words: H3N2 subtype, swine influenza virus, *NS1* gene, phylogenetic analysis, nucleotide

INTRODUCTION

Swine Influenza Virus (SIV) is one of the pathogens which cause respiratory diseases accompanied with coughing and sneezing in pigs (Olsen *et al*., 2006). The virus is an important pathogen not only from the viewpoint of animal health but also from that of public health (Olsen *et al*., 2006; Thacker and Janke, 2008; Loeffen *et al*., 1999). The segmented nature of genomes of Influenza A viruses allows the exchange of the gene segments when a pig is infected simultaneously with various viruses. So, pigs play a mixing vessel role to produce a novel influenza virus by genetic reassortment (Webster *et al*., 1992) as they have dual susceptibility to both human and avian influenza viruses (Kida *et al*., 1994; Takemae *et al*., 2011).

The pig industry in Chongqing has been expanding rapidly as one of the major livestock industries. Studies had showed that SIVs circulated in China from 2000 to 2010 and had acquired genetic diversity due to multiple introductions of Porcine Respiratory and Reproductive Syndrome Virus (PRRSV), Eurasian avian-like swine and human viruses. In addition, transmission of human viruses to pig (Takemae *et al*., 2008) or vice versa (Komadina *et al*., 2007) was also, suggested. However, ecology and the prevalence of SIVs in the Chongqing pig population have not been well characterized. Here, forty nine strains of H3N2 subtype swine influenza viruses identified by specific Reverse Transcription-PCR Method were isolated from the swine in Chongqing, China during the 2010 to 2011. *NS1* genes of these strains were sequenced and analyzed with the representative strains published in GenBank. Virological analyses in this study provided significant information needed to establish a strategy for SIV monitoring in pig farms.

MATERIALS AND METHODS

Viruses: Forty nine AIV strains tested in this study were isolated from unhealthy pigs in the vaccinated swine flocks in the Rongchang, Dianjiang, Dazu and Shizhu cities in Chongqing, China during the period of December 2010 to November 2011. The pigs showed respiratory symptoms and with 5-10% mortality in the isolated cases. All viruses were propagated in 10 days old specific pathogen free embryonated chicken eggs and were identified to be the H3N2 subtype by the Reverse Transcription-PCR (RT-PCR) Method. Specific pathogen free embryonated chicken eggs were provided by Shandong Sai's poultrystuffs Group Co., Ltd. Shandong, China.

RNA extraction and 1-Step RT-PCR: Viral RNA extraction of the harvested allantoic fluid was completed using minBEST Viral RNA/DNA Extraction kit (Takara Biotechnology, Dalian, China) according to the manufacturer's instructions. Reverse transcription-PCR was carried out by PrimeScript One-Step RT-PCR kit (Takara Biotechnology) in a 25 μL reaction volume containing 20 μL of RT-PCR PreMix (Takara Biotechnology; reaction buffer, deoxynucleoside triphosphate, 2 μL of enzyme mix), 2 μL of extracted viral

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RNA and the specific primer pair (H3N2P1: 5'- GTCTCAG GGAGCAAAAGCAGGGAG-3' and H3N2P2: 5'- GTATTA GTAGAAACAAGGGTGTTTT-3'). Reverse transcription and amplification were performed as 1 cycle of 50°C for 10 min, 95°C for 4 min followed by 35 cycles of denaturation at 94°C for 50 sec, annealing at 54°C for 50 sec and extension at 72°C for 60 sec, respectively with a final 10 min extension step at 72°C. The PCR products were cloned into a pMD18-T vector (Takara Biotechnology) for later sequencing (Yingjun Biotechnology, Shanghai, China).

Genetic variability and phylogenetic analysis: Nucleotide and the derived amino acid sequences of the 49 obtained *NS1* genes were compared with other H3N2 strains, A/swine/Heilongjiang/1/05(H3N2)) and A/swine/Fujian/F2/2007 (H3N2). The multiple alignment was carried out using DNAStar (Version 4.0) sequence analysis software (DNAStar Inc., Madison, WI). The phylogenetic tree was constructed with 49 acquired NS amino acid sequences, the representative NS sequences from the GenBank database including the A/swine/Guang Dong/811/2006 (H3N2), A/swine/ Colorado/1/1977(H3N2), A/Netherlands/233/1982(H3N2)) were represented for grouping using the MEGA 4.1 Software with Neighbor-Joining Method (Wu *et al*., 2010). In addition, other appropriate known strains in the world were also contributed to the tree.

RESULTS AND DISCUSSION

Nucleotides sequence: >NS(CQ isolated)_seq GTCTCAG GGAGCAAAAGCAGGGAGACAAAGACATAATGGA TTCCAACACTGTGTCAAGTTTTCAGGTAGATTGCT TCCTTTGGCATGTCCGAAAACAAATTGTAGACCA AGAACTAGGTGATGCCCCATTCCTTGATCGGCTTC GCCGAGATCAGAAGTCCCTAAGGGGAAGAGGCAG CACTCTCGGTCTAGACATCGAAGCAGCCACCCATG TTGGAAAGCAAATAGTAGAGAAGATTCTGAAGG AAGAATCTGATGAGGCGCTTACAATGACCATGGC CTCCACACCTGCTTCGCGATACATAACTGACATGA CCACTGAGGAATTGTCAAGAGACTGGTTCATGCTA ATGCCCAAGCAGAAAGTGGAAGGACCTCTTTGCA TCAGAATAGACCAGGCAATCATGAATAAGAACA TCATGTTGAAAGCGAATTTCAGTGTGATTTTTGAC CGGCTAGAGACCCTAATATTACTAAGGGCTTTCAC CGAAGAGGGAGCAATTGTTGGCGAAATCTCACCA TTGCCTTCTTTTCCAGGACATACTATTGAGGATGT CAAAAATGCAATTGGGGTCCTCATCGGAGGACTTG AATGGAATGATAACACAGTTCGAGTCTCTAAAAC TCTACAGAGATTCGCTTGGGGGAGCAGTAATGAGA ATGGGGGACCTCCACTTACTCCAAAACAGAAACG GAAAATGGCGAGAACAACTAGGTCAAAAGTTCG AAGAGATAAGATGGCTGATTGAAGAAGTGAGAC ACAGACTGAAAACAACTGAGAATAGTTTTGAGC AAATAACATTCATGCAAGCCTTACAGCTACTATT TGAAGTGGAACAGGAGATAAGAACTTTCTCGTTT CAGCTTATTTAATGATAAAAAACACCCTTGTTTC TACTAATAC

Phylogenetic analysis: To understand the genetic relationship of the tested strains with the representative isolates, the phylogenic trees were produced by using the deduced amino acid sequences of the *NS* gene. As is shown in Fig. 1 the phylogenetic relationship of strains at different times and geographical regions displayed complexity and diversity.

In the present study, the phylogenetic and molecular property of the H3N2 SIV strains isolated at different regions in Chongqing, China during 2010 to 2011 were described. It may present an even larger concern considering the recent outbreaks of influenza in spite of the low virulence because these strains were of human-like receptor specificity. When a control policy is

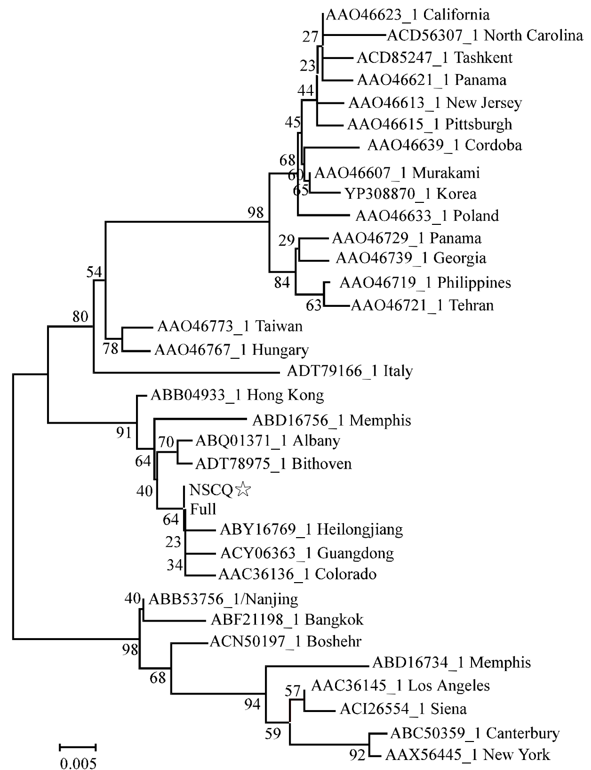

Fig. 1: The phylogenetic tree of H3 Swine Influenza Viruses (SIV) isolated in Chongqing, China during 2010 to 2011 based on the viral hemagglutinin sequences. The tested strains in this figure were marked with star

established, the genetic disparity between the current prevalent strains and the out of date other strains should be taken into account. This study would lay a foundation for selecting the candidate virus strain for vaccination production.

CONCLUSION

The results indicated a similarity among the virus strains isolated in the close time stage whereas the strains isolated maintained more difference with other strain. It is showed that the *NS1* genes of these strains and the A/swine/Guang Dong/811/2006(H3N2) and Influenza A virus (A/swine/Colorado/1/1977(H3N2)), A/Netherlands/233/1982(H3N2)), A/swine/Heilongjiang/1/05(H3N2)) and A/swine/Fujian/F2/2007(H3N2) displayed nucleotide homologies nearly ranging 99%. These results suggested that the strains might be derived from human influenza.

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