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Short communication

Identification of an H6N6 swine influenza virus in southern China

Guihong Zhang^{a,1,*}, Weili Kong^{a,1}, Wenbao Qi^{a,1}, Li-Ping Long^b, Zongxi Cao^a, Liangzong Huang^a, Haitao Qi^a, Nan Cao^a, Wenhua Wang^a, Furong Zhao^a, Zhangyong Ning^a, Ming Liao^{a,*}, Xiu-Feng Wan^{b,*}

^a College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

^b Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA

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ABSTRACT

This is the first report of avian-like H6N6 swine influenza virus from swine in southern China. Phylogenetic analysis indicated that this virus might originate from domestic ducks. Serological surveillance suggested there had been sporadic H6 swine influenza infections in this area. Continuing study is required to determine if this virus could be established in the swine population and pose potential threats to public health.

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Influenza A virus can infect various hosts, including migratory waterfowl, resident birds, horses, swine, dogs, sea mammals, and humans. Among these species, swine serve as an important component of the human–animal interface and play an important role in influenza evolution and ecology. The pig is proposed as a “mixing vessel” and an intermediate host for generating novel influenza reassortants including 1968, 1957, and 2009 pandemic influenza viruses (Scholtissek, 1990). The 2009 H1N1 pandemic virus is a swine-origin reassortant: HA, NP and NS segments from classical swine (North American) lineage; PB2 and PA segments from avian (North American) lineage; PB1 segment from human seasonal H3N2; and NA and MP segments from Eurasian swine lineage (Dawood et al., 2009).

H6 subtype of influenza A virus has been identified widely in migratory waterfowl (Munster et al., 2007) as well as the resident birds such as chicken, quail, pheasant, domestic geese and ducks (Chin et al., 2002; Lee et al., 2006; Woolcock et al., 2003). Experimental infection demonstrated that H6 avian influenza virus (AIV) can replicate in the upper respiratory track and cause mild clinical symptoms in humans (Beare and Webster, 1991). Serological surveillance suggested that H6 AIVs could infect veterinarians exposed to domestic birds (Myers et al., 2007). This subtype caused also an outbreak in chicken farms in South Africa (Abolnik et al., 2007). In southern China, new lineages of H6 AIVs have been circulating in

the domestic duck (Cheung et al., 2007; Chin et al., 2002; Huang et al., 2010). We here report an H6N6 swine influenza virus (SIV) from sick pigs on a small farm in southern China, and our serological surveillance suggested there were sporadic H6 swine influenza infections at four swine farms in this area. The results from this study indicate that H6 influenza A virus could use swine as an intermediate host before adapting to and causing infections in humans.

Southern China is designated as a putative influenza epicenter (Shorridge and Stuart-Harris, 1982). In order to monitor the epidemics of SIVs and to identify the potential swine influenza threats in southern China, we performed an active surveillance in the swine population in southern China. A total number of 847 samples were collected from 23 swine farms across Guangdong, Guangxi and Hainan Provinces, China. These samples include nasal swabs and lungs from sick pigs showing symptoms of coughing and/or rhinorrhea. Among these samples, 35 were shown to be RT-PCR positive for influenza A viruses. The RT-PCR tests were conducted based on the WHO Manual on Animal Influenza Diagnosis and Surveillance. Both nasal swabs and lung tissue homogenate were inoculated into 10-day-old specific pathogen free embryonated chicken egg, through the allantoic route, and incubated at 35 °C for 72 h. Subtype identification was conducted through standard hemagglutination inhibition and neuraminidase inhibition assays. Nine strains of SIVs were isolated: two H1N1, one H1N2, one H9N2, four H3N2, and one H6N6.

Due to the potential threats of H6 influenza A virus to public health, this study focused on the H6N6 subtype, A/swine/Guangdong/K6/2010 (H6N6). The genome of this H6N6 SIV was sequenced fully as described previously (Li et al., 2010), with the GenBank Accession Nos. HM800947 and HM804474 to HM804480.

* Corresponding authors.

E-mail addresses: guihongzh@scau.edu.cn (G. Zhang), wan@cvm.msstate.edu (X.-F. Wan).

¹ These authors contributed equally.

Phylogenetic analysis showed that H6N6 SIV originated from domestic aquatic birds. The HA of this H6N6 SIV belongs to the Group II lineage of H6 AIVs circulating in southern China (Fig. 1A). The NA, PB1, PA, NP, NS, and MP from H6 AIVs were also from waterfowl (Fig. 1B and Supplementary Fig. S1). The PB2 gene was phylogenetically close to those in H5N1 highly pathogenic avian influenza viruses (HPAIVs). The nucleotide sequence identity between each segment of A/swine/Guangdong/K6/2010 (H6N6) and its potential progenitor identified in the public database varied from 92.5% to 98.8%.

The protein sequence analysis on the HA gene of A/swine/Guangdong/K6/2010 (H6N6) showed that this virus is lacking polybasic amino acids in the connecting peptide. The receptor binding sites, including 130-loop, 190-helix, and 220-loop, of A/swine/Guangdong/K6/2010(H6N6) were similar to those in its

progenitor H6 AIVs. However, compared with those in its progenitor, there were two mutations, A222V and G228S, in A/swine/Guangdong/K6/2010(H6N6). A G228S mutation of H5N1 influenza virus was shown to expand the viral binding profile from ciliated cells to both non-ciliated cells and ciliated cells (Ayora-Talavera et al., 2009). Both H2 and H3 human viruses have the same substitutions, Q226L and G228S, whereas those with Q-226 and G-228 (avian and equine viruses) recognize SAa2, 3Gal (Connor et al., 1994). However, further experiments will be required to study whether A222V and G228S change the cell and host tropisms of this H6N6 SIV.

In order to detect the prevalence of such H6N6 in swine population, a total number of 475 swine serum samples were collected from sick pigs at 11 farms at Guangdong, Guangxi, and Hainan Provinces, China. These sera were tested against H6

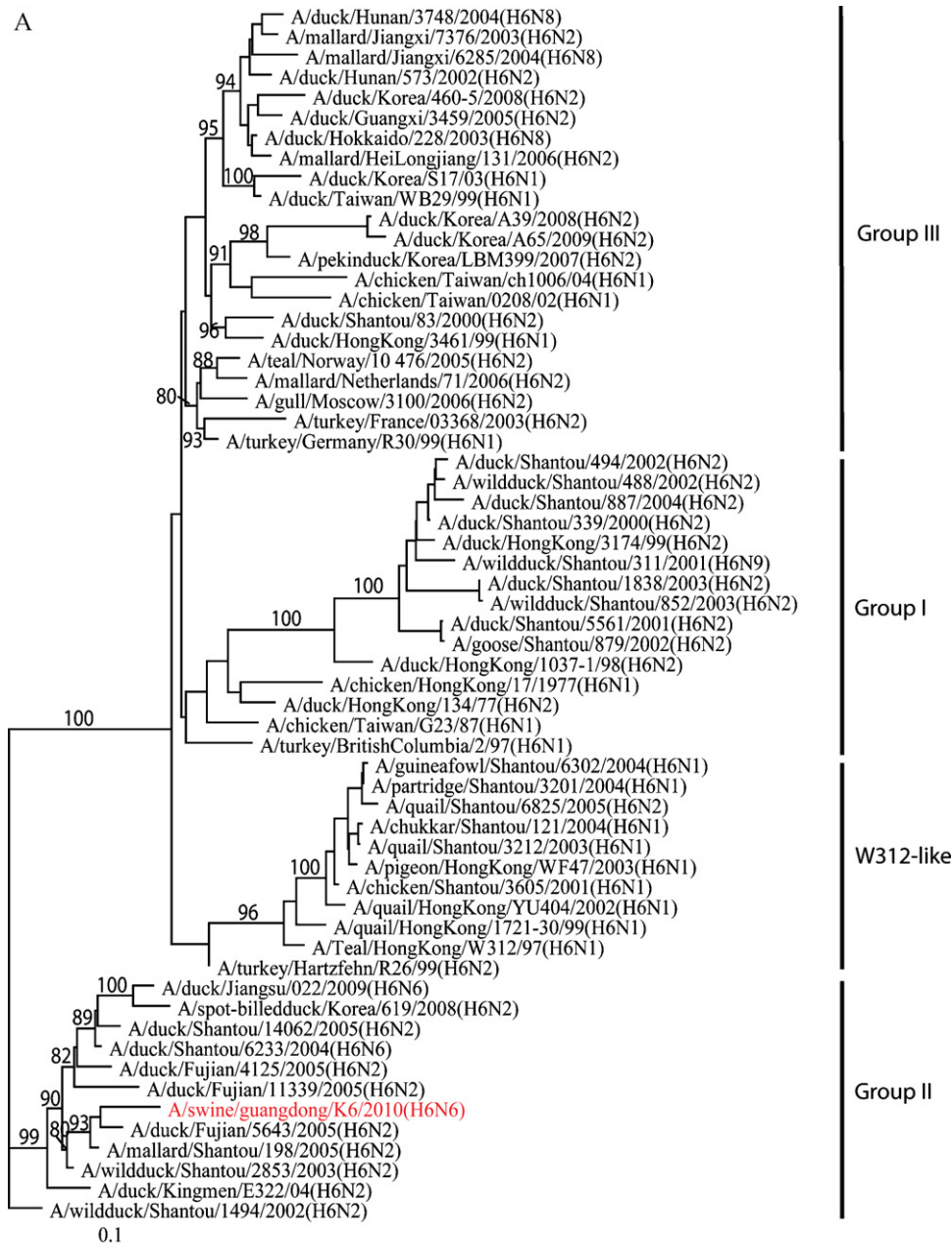


Fig. 1. Phylogenetic analysis of H6N6 swine influenza virus isolated from southern China. (A) The phylogenetic tree for HA gene; (B) the phylogenetic tree for NA gene. The phylogenetic trees were constructed using maximum likelihood implemented in GARLI version 0.96, and the bootstrap values were generated using neighborhood joining methods implemented in PAUP* with 1000 replications.

B

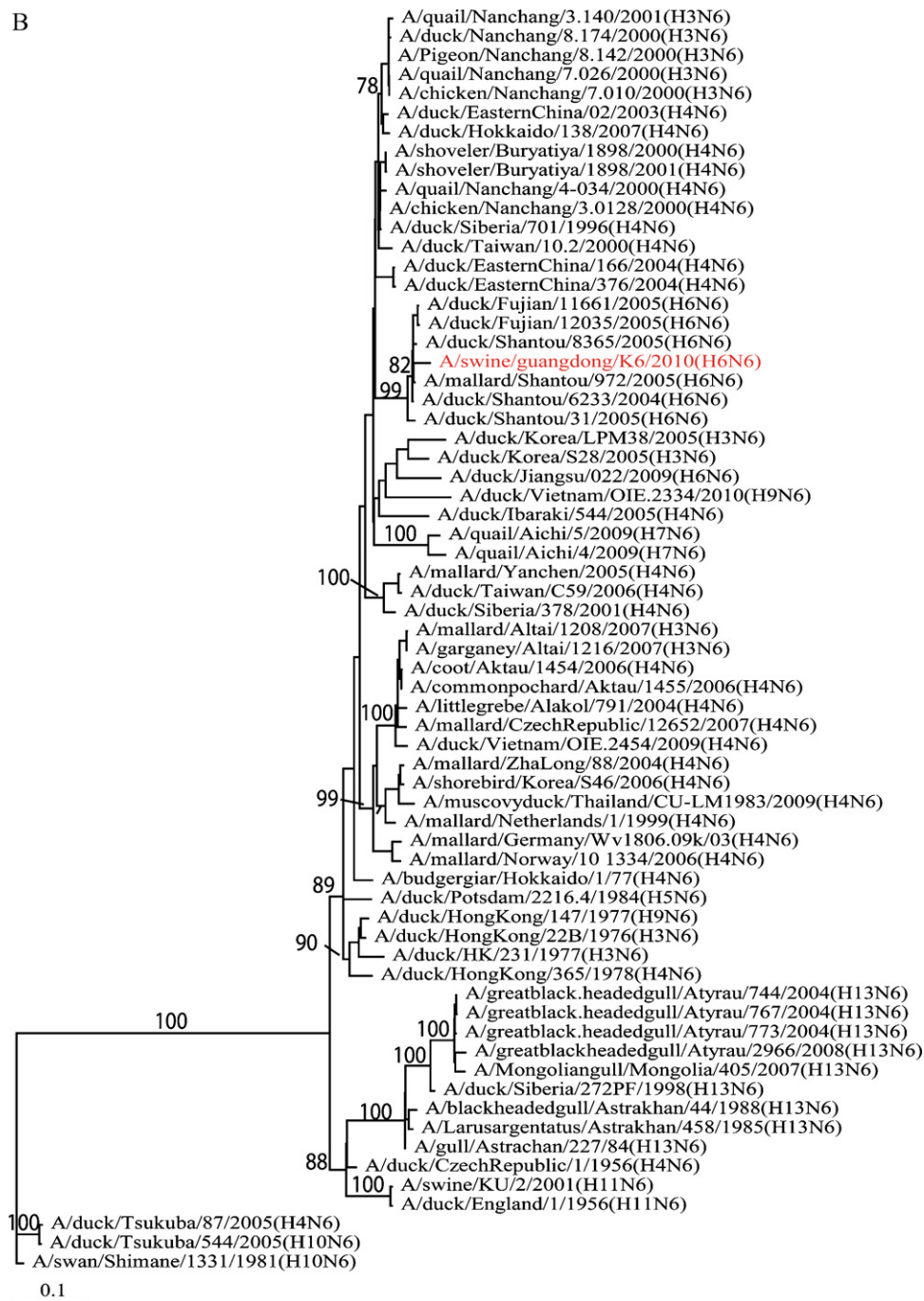


Fig. 1. (Continued).

subtype using hemagglutination inhibition assay, and all sera with a titer equal or more than $4\log_2$ were defined as positive. Our results demonstrated that 16 of these samples (3.4%) were positive against H6 SIV. These positive samples were collected from four swine farms located at the suburb areas in Shenzhen and Yangjiang of Guangdong province, and Guining and Guigang of Guangxi province.

In the past several decades, many influenza epidemics and pandemic strains were reported to emerge from southern China. Both 1957 and 1968 pandemic influenza emerged from this area. The precursor of the ongoing H5N1 HPAIVs was identified also in Sanshui, Foshan, a rural area in southern China (Wan, 1998). Since then, various genotypes of these H5N1 viruses have been identified

in this area. Since 2007, avian origin H3N2 canine influenza viruses have been identified also in the dogs in southern China (Li et al., 2010). The report of this H6N6 SIV in southern China further confirmed this area is critical for emergence of novel influenza A viruses.

Identification of H6N6 SIV in the domestic swine suggested that the epidemic of H6 SIV has been expanding from resident birds to the swine population. Our serological surveillance demonstrated a 3.4% of H6 infection rate in this area. Due to the potential of H6 influenza A viruses causing human infection, it is possible that more transmissible H6 viruses may be generated in swine through mutations and reassortments. Continuous monitoring of this virus in swine population is needed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.02.023.

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