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Characterization of H3N2 Influenza Viruses Isolated From Pigs in Southern China

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Human-like H3N2 influenza viruses have repeatedly transmitted to domestic pigs in different regions of the world, but it is still not certain whether any of those variants have become established in pig populations. The detection of different subtypes of avian influenza viruses from pigs makes it an ideal candidate for the genesis of a possible reassortant virus with both human and avian gene segments. However, whether pigs could act as a "mixing vessel" for a possible pandemic virus remains unanswered. Long-term influenza surveillance in pigs in southern China revealed that H3N2 influenza viruses were regularly detected from domestic pigs from 1998 to 2003. Antigenic analysis of representative strains revealed that two distinguishable groups of H3N2 influenza viruses were present in pigs during this period: a contemporary human-like viruses (represented by Sydney/5/97), and Port Chalmers/1/73-like (PC-like) viruses. Phylogenetic analysis of the representative strains confirmed those two groups. In general, the PC-like viruses were most closely related to those H3N2 reassortants recognized from European pigs since the mid-1980s, while the remaining isolates were most closely related to those contemporary human H3N2 viruses. It is interesting to note that one PC-like isolate contained a classical swine H1N1-like NP gene, Sw/HK/1197/02, suggesting that after introduction to pigs in southern China the European swine H3N2 virus further reassorted with local swine virus. The contemporary human-like H3N2 viruses isolated from pig appeared to have resulted from repeated introduction from humans to pigs. Interestingly, one isolate (Sw/HK/NS1128/03) clustered with those human isolates detected in the early 1990s. These findings suggesting that some recent human H3N2 variants may be maintained long-term in pig populations in southern China. The present study provides updated information on the role of pigs in the interspecies transmission and genetic reassortment of influenza viruses in this region.

Introduction

Pigs are considered an important host of influenza A virus as they might be associated with the generation of human pandemic influenza strains [1]. Historically, two human pandemic viruses, H1N1 in 1918 and H3N2 in 1968, were detected almost simultaneously in humans and pigs [2, 3]. Even though pandemic H3N2 virus was initially detected in humans and the full genome of the 1918 H1N1 pandemic virus was recently decoded [4], it is still unknown whether this virus

was first introduced into humans or pigs before it became a human pandemic strain. Currently, H1N1, H1N2 and H3N2 influenza viruses co-circulate in pigs in different regions. All of these viruses resulted from either interspecies transmission or reassortment events [5-7]. The Sydney-like H3N2 variants from pigs in the USA in 1998 were double and triple reassortants containing viral genes of avian, human and swine origins [8], highlighting the complex and dynamic influenza ecology in pig populations. Our long-term surveillance also revealed that H1N1, H1N2 and H3N2 subtypes viruses also co-circulated in pigs in southern China [9]. Here we present the findings of genetic and antigenic characterization of swine H3N2 influenza viruses isolated from 1998 to 2003.

Results

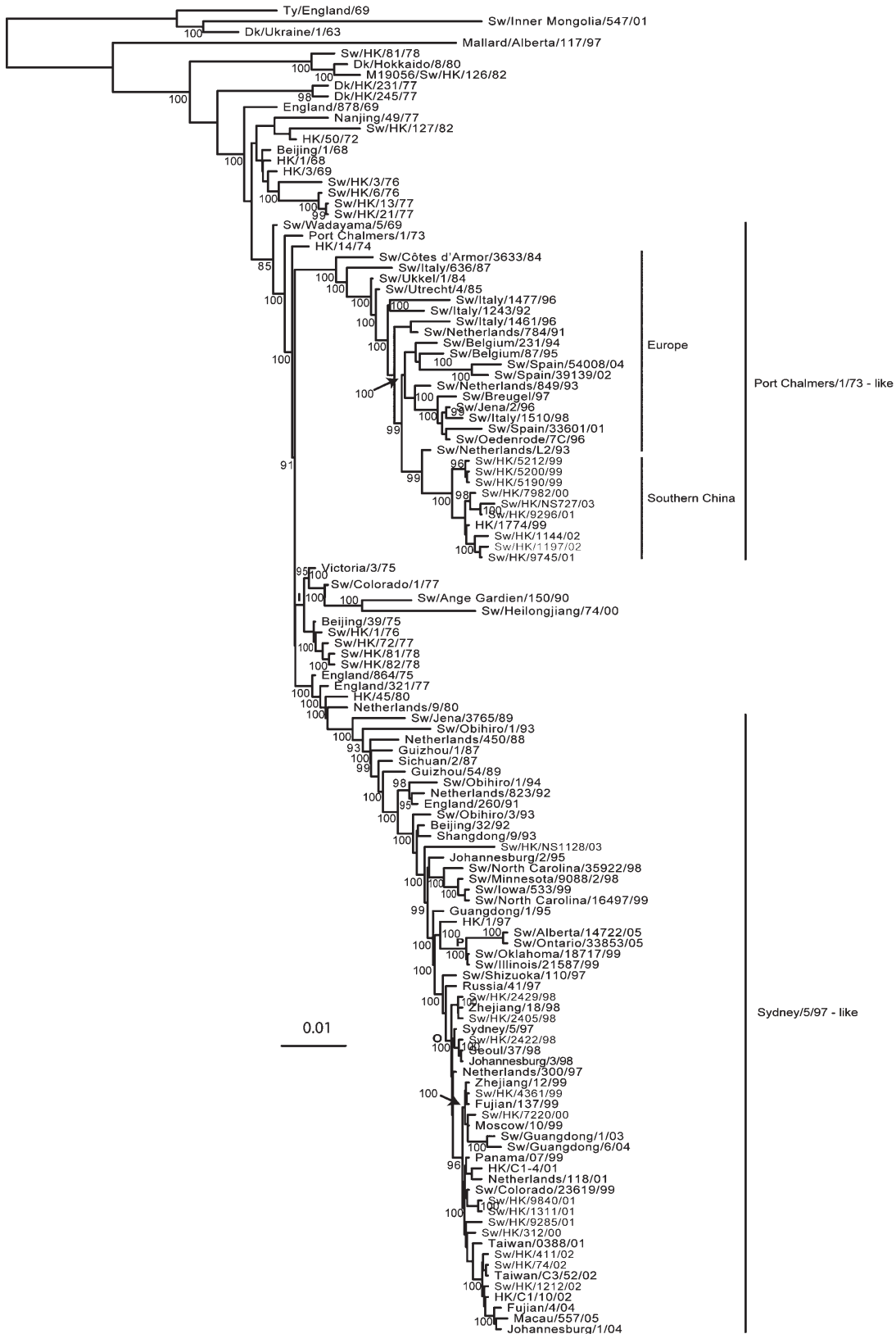
Genetic analysis showed that two sublineages of H3N2 influenza viruses were co-circulating in pigs in southern China. One sublineage of viruses, PC-like viruses, was closely related to those viruses prevailing in European pigs, while the other sublineage consisted of contemporary human-like variants resulting from multiple interspecies transmissions from humans to pigs. Epidemiological and genetic results also suggest that the PC-like H3N2 variants may have been introduced into pigs in this region via imported pigs from Europe in the mid-1990s.

Prevalence of swine influenza viruses in southern China. During the 8-year period of virological surveillance, 535 influenza viruses were isolated from 22,562 nasal and tracheal swabs (isolation rate 2.4%) from January 1999 to September 2006. Influenza virus in pigs was prevalent year-round but an increased isolation rate of H1 subtype virus was usually observed during the winter season. Ninety-nine of those isolates were H3N2 subtype, and 288 were H1N1 subtype viruses. The remaining 157 isolates were identified to be H1N2 viruses that were also detected each year during the surveillance period. In general, H1 subtype virus accounted for an increasing proportion of the overall number of isolates each year.

Phylogenetic analysis of the surface genes. Phylogenetic analysis of the H3N2 swine hemagglutinin (HA) genes revealed two distinct groups. The PC-like swine viruses formed a monophyletic clade with viruses isolated from swine in Europe since 1984 (Fig. 1). The PC-like viruses in southern China show high genetic similarity, forming a sister group to the European swine viruses isolated between 1994 and 2004, Sw/Netherlands/L2/93 in particular. Therefore, these viruses appear to have been introduced into pig populations in southern China from a Sw/Netherlands/L2/93-like virus (Fig 1). After their introduction into this region during 1997 these viruses appear to have persisted in pigs in southern China and have been detected in HK from 1999 to 2003. Phylogenetic analyses of the HA gene also shows that the Sydney-like swine H3N2 viruses that were isolated between 1998 and 2002 are closely related to several human viruses as well as the annual vaccine strains, indicating that these viruses were introduced into pigs several times from the human population. These results provide evidence for multiple introductions of H3N2

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Figure 1. Phylogenetic relationships of the HA genes of representative influenza A viruses isolated in pigs from southern China. Trees were generated by neighbor-joining method in the PAUP* program (Bayesian analysis revealed similar relationships.) Numbers at branches indicate bootstrap values. The tree was constructed based on nucleotides 161-952 of the HA gene and rooted to A/Duck/Alberta/28/1976 (H4N6). Scale bar, 0.1 substitution.



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viruses from humans to pigs. However Sw/HK/NS1128/03 was most closely related to the earlier human strains (e.g. Shandong/9/93), indicating that some human H3N2 variants could be maintained in pig populations for a long period. These phylogenetic relationships are consistent with the antigenic reaction pattern. A similar phylogenetic relationship was observed in the neuraminidase (NA) gene tree, except for Sw/HK/NS1128/03, which clustered with recent swine H1N2 isolates (Sw/Zhejiang/1/04) in China.

Phylogenetic analysis of the internal genes. Phylogenetic analysis of the six internal genes showed that the H3N2 viruses from pigs in southern China have undergone reassortment. In the PB2 gene tree, 11 representative H3N2 isolates clustered into three different lineages. The PB2 gene of representative PC-like swine viruses clustered with European swine viruses that appear to have originated from an avian source. Except for one virus, all Sydney-like viruses clustered with recent human H3N2 viruses, indicating a human origin. The PB2 gene of one of the Sydney-like viruses, Sw/HK/NS1128/03, belonged to the classic swine H1N1 lineage. Similar phylogenetic relationships was observed in the PB1, PA and M gene trees. However, in the NP gene tree, PC-like viruses belonged to a recent human lineage, while Sydney-like viruses belonged to an avian lineage, except for the virus Sw/HK/1197/02. This virus and Sw/HK/NS1128/03 belonged to the classic swine H1N1 lineage. In the NS gene tree all PC-like viruses grouped with the European swine lineage, while all Sydney-like viruses joined a recent human lineage. Taken together, four different reassortants, or genotypes, of H3N2 viruses were recognized. Two of them were the European swine (PC-like) virus, and PC-like virus but with a NP gene from classical swine H1N1 virus. The other two genotypes included contemporary human-like, and a triple reassortant (Sw/HK/NS1128/03) that contained surface genes from an early 1990's human-like H3N2 virus, but with five internal genes from classical H1N1 swine virus and an NS gene that of avian origin.

Table 1. Gene origins of swine H3N2 influenza viruses from southern China from 1998 to 2003^a.

Viruses	Subtype	Lineage	Gene									Genotype
			PB2	PB1	PA	HA	NP	NA	M	NS		
Sw/Germany/2/81	H1N1	Av	Av	Av	-	-	Av	-	Av	Av	Av	Av
Sw/HK/168/93	H1N1	Av	Av	Av	Av	Av	Av	Av	Av	Av	Av	Av
Sw/HK/5190/99	H3N2	PC	Av	Av	Av	Hu	Av	Hu	Av	Av	Av	Double(Hu-Av)
Sw/HK/7982/00	H3N2	PC	Av	Av	Av	Hu	Av	Hu	Av	Av	Av	Double(Hu-Av)
Sw/HK/9296/01	H3N2	PC	Av	Av	Av	Hu	Av	Hu	Av	Av	Av	Double(Hu-Av)
Sw/HK/1197/02	H3N2	PC	Av	Av	Av	Hu	Csw	Hu	Av	Av	Av	Triple(Hu-Csw-Av)
Sw/HK/NS727/03	H3N2	PC	Av	Av	Av	Hu	Av	Hu	Av	Av	Av	Double(Hu-Av)
Sw/HK/2405/98	H3N2	SY	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu
Sw/HK/4361/99	H3N2	SY	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu
Sw/HK/7220/00	H3N2	SY	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu
Sw/HK/9285/01	H3N2	SY	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu
Sw/HK/1212/02	H3N2	SY	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu	Hu
Sw/HK/NS1128/03	H3N2	SY	Csw	Csw	Csw	Hu	Csw	Hu	Csw	Av	Av	Triple(Hu-Csw-Av)

Conclusions

Antigenic and phylogenetic analysis of swine H3N2 influenza viruses in southern China from 1998 to 2003 provided evidence of persistence of both early and contemporary H3N2 variants in pig populations. Results also revealed multiple interspecies transmissions of influenza viruses from human to pig and subsequent reassortment events, particularly with internal genes from avian lineages in this region. In the present study, PC-like viruses consistently formed their own clades for each segment except the NP gene of Sw/HK/1197/02. This indicates that most of the PC-like viruses may be derived from a single reassortant progenitor virus from European pigs imported into China as breeding stock, which subsequently spread among pigs, rather than as the products of multiple, independent reassortment events. Sydney-like viruses were from the contemporary human lineage and probably arose from multiple introduction events into pig, rather than being established in this host. Our findings demonstrate continuing interspecies transmission and reassortment events in pigs in southern China that naturally increase the possibility for pigs to be an important host for generating potential pandemic strains.

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