Epidemic Status of Swine Influenza Virus in China

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Abstract As one of the most significant swine diseases, in recent years, swine influenza (SI) has had an immense impact on public health and has raised extensive public concerns in China. Swine are predisposed to both avian and human influenza virus infections, between that and/or swine influenza viruses, genetic reassortment could occur. This analysis aims at introducing the history of swine influenza virus, the serological epidemiology of swine influenza virus infection, the clinical details of swine influenza, the development of vaccines against swine influenza and controlling the situation of swine influenza in China. Considering the elaborate nature of swine influenza, a more methodical surveillance should be further implemented.

Keywords Swine influenza epidemiology · Vaccine · Prevention

Introduction

Swine influenza (SI) is an acute respiratory tract disease in pigs, characterized by high fever, dyspnea, depression,

coughing, sneezing and weight loss. It has become a crucial viral pathogen in the pig-raising industry and has been a serious threat to human health. Swine influenza virus (SIV) was recognized to be a causative agent in the porcine respiratory disease complex (PRDC) [1]. SIV, an influenza A virus, is an enveloped negative-stranded RNA virus with a segmented genome including eight gene segments. The virus has never stopped changing and hence producing new viral subtypes. Such mutations include point mutation that lead to gradual change (genetic drift) and gene reassortment which eventually result in a dramatic change (genetic shift). Presently, there are various subtypes of swine influenza virus in China. H1N1, H3N2 and H1N2 were the predominant genetic subtypes of the virus in pigs. H9N2 SIVs, which have been discovered since 2003 and have become epidemic in swine population. Other subtypes have also been isolated in swine, such as H3N8, H4N8, H5N1 and H6N6. These subtypes have shown up in various provinces, including Hubei, Anhui, Fujian and Guangdong provinces. In 2010 the widely known and dreaded pandemic (H1N1) 2009 virus also infected the pigs in Hubei and Jiangsu, which caused a dreadful disaster to public health and affected China's economy dramatically.

China is not only the biggest country of pigs and pork production, but also has the largest market for the consumption of pork in the world. Pigs are afflicted with SIV along with other mutations of virus. SI starts by first reducing the affected pig's immunity which makes the pig prone to secondary and mixed infection with porcine reproductive and respiratory syndromes virus (PRRSV), porcine circovirus type virus 2 (PCV2), classical swine fever virus (CSFV), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* [2, 3]. These pathogens interact with each other and generate PRDC.

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History and Serological Epidemiology of SIV in China

Throughout the world, the first swine influenza virus A/swine/Lowa/15/31 (H1N1) was identified by Shope who was a researcher in the Rockefeller Institute, America [4]. At the same time, A/swine/Lowa/15/31 (H1N1) became the representative virus of classical swine influenza virus (H1N1). The classical swine influenza virus caused a high mortality among pigs in Chinese coastal cities between the years 1918 and 1919 [5]. Meanwhile, the pandemic H1N1 virus triggered an outbreak of diseases in human beings in 1918. Surprisingly, evolutionary studies revealed that the classical swine H1N1 and the 1918 pandemic H1N1 viruses share a common ancestor and have similar characteristics [6, 7]. The classical swine H1N1 influenza was first reported in 1991 in Beijing [8]. Genbank and serological studies of the swine influenza virus, showed that both the classical swine H1N1 and European avian-like H1N1 were widely distributed in many regions, including Beijing, Henan, Shandong, Shanghai, Jiangxi, Guangdong, Guizhou, Taiwan and Hong Kong. European avian-like H1N1 and human-like H1N1 were also discovered in the pig population. However, it is rare to find human-like H1N1 viruses springing up in China [9], nevertheless, in 2010 avian-like swine influenza A (H1N1) viruses infected humans in China [10]. This phenomenon suggested that after long-term adaptation in pigs, the avian-like A (H1N1) SIVs have already been replicated in human beings. During 2009–2011, the pandemic (H1N1) 2009 virus was isolated in Jiangsu, Hubei and Guangdong from swine [11–13]. The pdm/09-like virus seropositivity appeared in both Guangdong, representing 16.6 % (104/625) and Guangxi, representing 8.9 % (35/625). This demonstrated that the pdm/09-like virus had spread widely in the swine population in South China. As at now, the pandemic (H1N1) 2009 virus has not yet been found to be transmitted to humans from pigs. In 2010, a novel triple-reassortant H1N1 was first found in China, which infected pigs in Guangdong. This virus contained genes from the classical swine (NP, NS), human (PB1) and avian (HA, NA, M, PB2, PA) lineages [14]. In the same year, another novel virus with European avian-like surface genes and pdm/09 internal genes was found in Hong Kong pig herds [13, 15]. Reassorted pandemic (H1N1) 2009 was also found in pigs in Germany and United States [16, 17]. It is likely that the continuing prevalence of the pdm/09 virus in pigs can cause the genesis of novel swine reassortant viruses which has the potential of infecting human beings.

Other isoforms like H3N2 influenza viruses were discovered in pigs and led to a long-term infection. The H3N2 SIV could be divided into four types: wholly human-like H3N2 viruses, wholly avian-like H3N2 viruses, double-reassortant H3N2 viruses (DR H3N2), and triple-

reassortant H3N2 viruses (TR H3N2) [18, 19]. DR H3N2 and TR H3N2 include various reassortants between avian, human and swine that has resulted in interspecies transmission from human or avian to pigs. On the contrary, reassortant between mammal H3N2 viruses and avian H5 viruses were isolated in pigs of Jilin recently [20]. Owing to potential danger to human beings, the virulence and transmissibility of H3/H5 reassortant SIVs need to be evaluated urgently.

In 2004 the first H1N2 appeared in swine population in Zhejiang, which is a novel reassortant with human-like H3N2 virus and classical swine H1N1 virus [21]. Then North America H1N2 swine flu was also isolated in Guangxi and Guangdong in 2006 [22]. In 2010 the avian-swine-human triple-reassortant virus caused illness in Guangdong pigs. Based on analysis, as the swine was infected by foot and mouth disease, which triggered H1N2 influenza infections outbreak in Guangdong [23]. The three viruses (H1N2, H1N1, H3N2) are the three genotypes discovered in pigs in China but we still do not know the pathology and transmission of H1N2 SIVs in pigs.

From 2003 to 2010, 34 strains of H9N2 SIV were isolated from swine population in China [24]. According to phylogenetic analysis, these H9N2 SIVs were classified into 13 genotypes from A to M. Being the dominant virus, all of the H9N2 SIVs originated from avian influenza virus. Although H9N2 SIVs belong to lowly pathogenic avian influenza virus for pigs, avian H9N2 influenza A virus still repeatedly infect human beings [25–28]. Considering the risk of H9N2 viruses to humans, some avian H9 pandemic influenza could infect the ferret model, which poses a higher potential threat to human beings [29]. Sun et al. [30] found that the contemporary avian H9N2 influenza virus and pandemic H1N1 virus showed high genetic compatibility and could generate reassortants with higher pathogenicity than parental viruses, which indicates that H9N2 influenza virus could contribute to the evolution of viruses with an increased threat to public health by reassorting with the pandemic H1N1/2009 virus.

Two strains of H3N8 influenza virus were isolated from pigs in central China during 2005–2006, which came from European equine H3N8 influenza virus in the early 1990s. The infected pigs showed obvious respiratory disease symptoms including depression and coughing. *Streptococcus suis* serotype 2 was also isolated from the same pigs, which showed mixed infection. Several important substitutions that appeared in hemagglutinin (HA) amino acid sequence may affect its biological characteristics [31]. In 1989, avian influenza virus was isolated from sick horses, which caused up to 20 % mortality in some herds [32]. In 2010, the fourth outbreak of equine influenza virus appeared in mainland, China. It spread widely in many species including domestic horses, wild horses,

competition horses and donkeys from the different regions of China [33]. Since the horse race games have become more popular in China, it is necessary to pay more attention to equine influenza. Furthermore, from September to December 2011, 162 New England harbor seals died in an outbreak of pneumonia. Sequence analysis of postmortem samples revealed the presence of an avian H3N8 influenza A virus in those harbor seals [34].

In 2002, it was reported that pigs in southeastern China have been sporadically infected with H5 influenza viruses through neutralization test [35]. Within 2001–2003, two H5N1 avian influenza viruses were isolated from swine at Fujian in China. According to seropositive survey, it was also found that H5 influenza virus infected pigs [36]. However, other researchers results revealed no evidence of H5 infection with pigs in Fujian, when they surveyed swine populations in Fujian province in 2004 and 2007 for serological evidence of H5 viruses infection [37]. This seemed controversial. In 2005–2007, other researchers found that influenza A (H5N1) viruses have been transmitted multiple times to pig populations in Indonesia, but the infected pigs were lack of influenza-like signs. These indicated that influenza A (H5N1) viruses can replicate undetectedly for prolonged periods, facilitating avian virus adaptation to mammalian hosts [38]. In summary, H5N1 influenza virus does infect pigs.

In 2011, we found that wholly avian-like H6N6 and H4N8 swine influenza virus were directly transmitted from waterfowl or wild waterfowl to the pigs in China [24, 39]. Compared to those in their progenitors, there were two mutations, A222V and G228S, in H6N6. The G228S mutation, from previous reports of H5N1 influenza virus, was shown to expand the viral binding profile from ciliated cells to both non-ciliated cells and ciliated cells. In Guangdong, H6 avian influenza became further active in domestic ducks during the years 2000–2007, which have caused a transmission from avian to swine [40, 41].

In China, different swine influenza viruses are co-circulating in pigs. Novel SIVs could appear anytime through the gene reassortment or the gene mutation. It is possible that people would receive death threats by SIV and pigs could be infected by avian and human influenza virus. In other countries, the rare swine influenza virus was previously reported, including H1N7, H4N6, H3N3 and H7N2 [42–45] and some novel SIV included H3N1, H2N3, and H5N2 show up on occasion [46–48]. For distribution of swine influenza virus in China and other countries see Table 1. Avian influenza viruses, equine influenza viruses and swine influenza viruses are widespread enzootic, and they would generate some negative influences to both human and swine population in China.

Epidemic of SIV in China

Epidemiological Status

In China different subtypes of SIVs appeared in swine population. According to different reports, the average seropositive frequencies of subtypes H1, H3, H4, H5, H6, H7 and H9 were 3478/11,168 (31.1 %), 2900/10,139 (28.6 %), 9/110 (8.9 %), 77/5945 (1.3 %), 16/475 (3.4 %), 0/1440 (0 %) and 86/3619 (2.4 %), in China over a 10-year period (1998–2010) respectively [35, 39, 49]. A total of 946 strains of SIV were isolated in mainland China, Hong Kong and Taiwan. So far, there is no record of swine influenza virus in Macau.

Clinical Signs and Secondary Infection

As mentioned above, when the SIV infected the pigs, it exhibited the following: (i) it spread quickly in major pig-producing areas of China; (ii) it had obvious epidemic and transmission features when the outbreak occurred in one pig farm. When the affected cases were observed, the whole pig herd was infected within 1–3 days; (iii) the body temperature of affected pigs reached 40–43 °C, and the disease course was usually 7–10 days. Almost all pigs could get infection. However, death of the pigs was rarely seen; (iv) the disease affected pigs of all ages. However, the affected pig herd displayed high morbidity and low mortality. The morbidity was usually 100 %, and the mortality was about 1.88 %. Pregnant sows in different stages could be infected. Some infected pregnant sows suffered abortion and reproductive performance descended; (v) the affected pigs exhibited the clinical symptoms such as depression, anorexia, lethargy, recumbency and flocking together. Most of the diseased pigs showed obvious respiratory distress, i.e. sneezing, runny nose, coughing, dyspnea, increased eye secretion, conjunctivitis. Affected pigs could recover quickly at the rate as the outbreak of disease.

Generally, only infection of SIVs results in viral pneumonia. There is an obvious dividing line between normal and diseased lung tissues. The damaged area of the lung is diffusely firm and becomes purple. The lung lobe will be edematous and fibrin purulent exudates would appear in the respiratory tract. However secondary infection and co-infection are common than SIV mono-infection. Co-infection with other pathogens includes PRRSV, PCV2, CSFV, *M. hyopneumoniae*, *A. pleuropneumoniae* and *P. multocida*. Interaction between these pathogens causes porcine respiratory syndrome and results in more complicated infections [50–52].

Risk Factor of Swine Influenza

Many factors affected the occurrence of swine influenza including temperature, environment and breeding mode.

Table 1 Global identification of subtypes of swine influenza virus

Country	Region	Subtype	Name	Origin	Host
America	Lowa	H1N1	A/swine/Lowa/15/31	Avian	Swine
China	Beijing	H1N1	A/swine/Beijing/47/1991	Avian	Swine
China	Hong Kong	H1N1	A/swine/Hongkong/168/93 A/swine/Hongkong/176/93	Avian	Swine
China	Tianjin	H1N1	A/swine/Tianjin/01/04	Human	Swine
	Guangdong		A/swine/Guangdong/96/06		
	Henan		A/swine/Henan/01/06		
China	Guangdong	H1N1 triple-ressortant	A/swine/Guangdong/1/2010	Human/avian/swine	Swine
China	Zhejiang	H1N2 double-ressortant	A/swine/Zhejiang/1/04	Swine/human	Swine
China	Guangxi	H1N2 triple-ressortant	A/swine/Guangxi/13/06	Swine/human/avian	Swine
	Shanghai		A/swine/Shanghai/1/07		
	Guangdong		A/swine/Guangdong/1/10		
China	Taiwan	H3N2	A/swine/Taiwan/7310/70	Human	Swine
China	Hong Kong	H3N2 triple-ressortant	A/swine/Hong Kong/126/82 A/swine/Hong Kong/127/82	Swine/human/ avian	Swine
China	Hong Kong	H3N2 double-ressortant	A/swine/Hong Kong/5190/99 A/swine/Hong Kong/5200/99	Human/avian	Swine
China	Guangdong	H3N2	A/swine/Guangdong/L1/11	Avian	Swine
China	Hong Kong	H9N2	A/swine/Hong Kong/9/98 A/swine/Hong Kong/10/98	Avian	Swine
China	Fujian	H5N1	A/swine/Fujian/1/01	Avian	Swine
China	Hubei	H3N8	A/swine/Chibi/01/2005	Equine	Swine
	Anhui		A/swine/Anhui/01/2006		
China	Guangdong	H6N6	A/swine/Guangdong/K6/11	Avian	Swine
China	Guangdong	H4N8	A/swine/Guangdong/K4/11	Avian	Swine
China	Hong Kong	2009/Pandemic	A/swine/Hongkong/NS1583/09	Swine/human/avian	Swine
	Nanchang		A/swine/Nanchang/3/2010		
	Jiangsu		A/swine/Jiangsu/38/2010		
	Guangxi		A/swine/Guangxi/1/2011		
United Kingdom	England	H1N7	A/swine/Eng/191973/92	Human/equine	Swine
Canada	Ontario	H4N6	A/Swine/Ontario/01911-1/99	Avian	Swine
Korea	N	H7N2	A/swine/KU/16/2001	Avian	Swine
Canada	Ontario	H3N3	A/swine/Ontario/42729A/01	Avian	Swine
America	Indiana	H3N1	A/swine/MI/PU243/04	Human/swine	Swine
America	Missouri	H2N3	A/swine/Missouri/4296424/2006	Avian/swine	Swine
Korea	N	H5N2	A/swine/Korea/C12/08 A/swine/Korea/C13/08	Avian Avian/swine	Swine

N not known

As is known to all, the seasonality is one of the key features of swine influenza.

Almost all limited epidemics have occurred in winter every year. When temperature changes abruptly, it could trigger the occurrence of swine flu infection especially in late autumn, early spring and winter. The rain and low temperature also play overwhelmingly important roles in the infection of influenza virus [53, 54]. With the development of breeding mode, pigs industries became more

large-scale and intensive than before, and it increased the chances of swine flu in open-air rearies [55]. On one hand this change satisfied the growing demands for pork; on the other hand it increased the risk of swine flu. The breeding mode line operations would give the opportunity for the SIV to survive and stay in swine population for long time. The disease sets in immediately with variation in humidity and temperature. It spreads rapidly and does not subside easily. There are two forms of the infection: (1)

Symptomatic: where the infected swine presents with the characteristic high fever, dyspnea, depression, coughing, sneezing and weight loss. (2) Asymptomatic: where the infected pigs don't have any obvious clinical symptoms. Whatever form of the infections, there is no doubt that the disease would cause significant economic losses.

Recently many AIVs were isolated from the pigs' population. Therefore, theoretically, the poultry poses a threat to healthy swine. Surprisingly, the presence of poultry on a farm was not discovered to increase the risk of swine influenza infection [56]. Instead of poultry, the authors suggested, mammalian pets such as cats and dogs in seropositive farms had already been found to increase the risk of swine infection by influenza viruses [57].

Vaccine Development of SIV in China

In China there is no professional commercialization of the vaccine to control the SI. To date, at home and abroad, inactive vaccine (H1N1+H3N2) was produced by Intervet International Gesellschaft Mit Beschränkter Haftung, which was not widely used in China. Many animal health companies have also been trying to develop the swine influenza vaccine, including inactive vaccine and virus-like particle. Recently, different strategies are under development to produce vaccines that provide better cross-protection for swine. A DNA vaccine against swine influenza encapsulated in poly (D,L)-lactic-co-glycolic acid (PLGA) microspheres or chitosan nanoparticles were tried. The results demonstrated that the PLGA microspheres or chitosan nanoparticles containing DNA vaccine can be used to achieve prolonged release of plasmid DNA. These results have laid a foundation for further development before ultimate industrial application. However the cost of novel vaccine is still an issue to be addressed [58, 59]. Some recombinant live pseudorabies virus, swinepox virus or adenovirus expressing HA and HA1, recombinant plasmid DNA vaccine expressing HA, multiple antigenic peptide vaccine were developed actively [60–62]. Besides, producing universal influenza vaccines based on M2 is a novel directed research [63]. Recombinant modified influenza viruses can be obtained with reverse genetics technology and provide a novel way to make modified live-attenuated virus vaccines. Modifications introduced into the viral NS1 gene via reverse genetics have resulted in attenuated influenza viruses with promising vaccine potential. Previous studies showed that different attenuated influenza viruses could induce robust humoral and cellular immune responses, and generated immune protection against challenge with wild-type virus, including homologous strains or heterologous strains [64–67]. The virus containing a modified HA was demonstrated to be highly

attenuated in swine and showed potential for use as a modified live-virus vaccine after intratracheal inoculation in pigs. However, intratracheal vaccination is not routinely used in the swine industry and need to be changed [68]. Before the modified live-attenuated virus vaccines are released into the marketplace, safety is a major concern. Although these trials indicated that these vaccines could induce effective immune protection to SIV infection in pigs or antibody response in mouse model, still none of them were practically applied in the field. In other countries, inactivated autogenous vaccines were applied widely, which was licensed by the USDA Center for Veterinary Biologics [69]. However, autogenously vaccines haven't been approved by the Government of China.

According to the media reports, Harbin Pharmaceutical Group has already succeeded in making an acquisition of swine influenza vaccine on the mainland of Pfizer Pharmaceuticals. It means that we would have domestic swine influenza vaccine in China in the near future. What is more, South China Agricultural University, Huazhong Agricultural University as well as Harbin Veterinary Research Institute have been dedicating themselves to the development of swine influenza vaccines, and have gained primary productions.

Control and Future Perspective of SI in China

The Importance of the Swine Influenza

In recent years the swine disease has become so complicated that the prevention of the disease is difficult for veterinarian and pig farm managers in China and other countries. Foot and mouth disease (FMD), porcine reproductive and respiratory syndromes (PRRS), porcine circovirus type 2 (PCV2), aujeszky's disease and swine fever are also threatening to the swine population, especially interacting with mixed infection or secondary infection. In addition, porcine diarrhoea including porcine transmissible gastroenteritis (TGE), porcine epidemic diarrhoea (PED), porcine rotavirus (PoRV) and porcine kobuvirus (PKV) caused an epidemic recently during 2010–2011. Porcine bocavirus (PBoV) and other related novel porcine parvoviruses have emerged in pigs since 2009 and then these pathogens were also detected in swine in China [70, 71]. All of these diseases have a huge impact on swine health. Of course, this is due to historical reasons and the environment in China. Because of the low mortality of swine flu, most people usually pay less attention to it than they do to other diseases. This idea is absolutely wrong. Swine flu causes immunodepression as a means of predisposing the pigs to infection not to even talk about its negative impact on public health. Pigs have long been thought of as a

“mixing vessel” where human and avian influenza virus reassortment could occur [72]. In 2009, pandemic H1N1 evolved over a long period before causing a global pandemic in humans, which was determined to be an uncommon reassortant virus including genes of SIV [73]. In addition, some SIVs including European avian-like H1N1 and triple reassortant swine influenza viruses infected human beings sporadically in the world [10, 74]. Whatever angle you view this problem, it is still worthy of paying more attention to swine flu. The government and researchers should conduct epidemiological monitoring and further master the molecular characteristics of swine flu.

Biosecurity Practices

Currently, there are no effective vaccines developed in China for preventing swine flu; the imported vaccines are not used widely in China. The clinical results of the imported vaccines have also not been well understood. Without additional understanding of the impacts of vaccination on viral evolution, these strategies, even under the best case scenario, would only slow down or delay epidemics. In the worst case, they may even facilitate the evolution of increased virulence. Clearly, understanding the ecological and evolutionary aspects of transmission, including the environment of a changing world, are critical methods to minimize the bad impacts of influenza on swine and public health. Whether pig vaccination has an effect on decreasing aerosol transmission and regional spread remains a mystery worthy of investigation. Biosecurity practices remain the primary means of preventing or minimizing transmission of influenza A virus in pigs and from pigs to other species. Before introducing replacement animals into farms, pigs should be kept in isolation facilities and tested SIV prior to releasing them into the units. Because swine flu can transmit through biological aerosols, air-filter would be considered to be installed in pig farms in order to prevent SI [75]. It was proved that there was a strong relationship between the number of pigs shedding influenza virus in nasal secretions and the generation of bioaerosols during the course of an acute infection [76].

Pigs are susceptible to avian and human influenza A viruses. The transmission of avian influenza viruses has a close relationship with the activities of birds. It is necessary, therefore, to constitute bird screens to stop birds from contacting with swine. Also, since dogs and cats can be infected with avian influenza virus, they should not be kept in pig farm [77, 78]. Being a veterinarian, it is fundamental to monitor pig farm worker for flu-like symptoms. No one working in pig farm is allowed to start the work without being vaccinated with the vaccine of seasonal flu periodically. If somebody gets a cold, they should caution the

other workers to be kept a long distance away from the feeding areas. In order to cut off the route of transmission of influenza A virus between pigs and human beings, some strict supervisory control methods should be put into practice, such as ensuring that any visitor is required to take a bath before entering and leaving the facilities, and people must wash their hands and arms with soap for 10–20 s before entering. Meanwhile, some effective protecting facilities like face masks, eye protection and gloves should be used during working procedure. These measures are necessary for preventing the spread of the influenza.

Considering other conditions, elimination of influenza viruses from swine herds has not been effectively applied in China. Although the elimination of PRV and CSFV has been enforced in some regions, epidemiological surveillance too should still be taken seriously in China, especially in South China. Regular monitoring of serological and virological data should be done through biological software. Large sample collections are helpful to implement molecular analyses for studying gene variation. Considering public health and the development of pig breeding, long-term efforts that are still required in the future.

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