An Epidemiological Study of Swine Influenza in south China

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Declaration

I declare this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Abstract

Swine influenza (SI) can result in a significant economic loss for the pig industry and potentially lead to pandemic influenza in humans. Although SI is prevalent in south China, the epidemiological characteristics of its occurrence in this area were not known prior to the study described in this thesis. This study was mainly conducted in Guangdong Province to: estimate the prevalence of SI; identify risk factors for SI infection in pig farms; assess the knowledge, beliefs and practices (KBP) of pig industry workers towards SI; describe the movement network of live pigs via the wholesale live pig markets; identify anthropogenic, meteorological and geographical factors associated with swine, human and avian influenza viral infection in pigs in south China; and provide evidence of the benefit of risk-based surveillance to address the pandemic influenza threat in south China.

A cross-sectional survey was conducted in 153 commercial pig farms in Guangdong Province. The farm-level prevalence of farmer-perceived SI during a six-month period was estimated to be 58% (95% CI: 48 - 68%). Statistically significant risk factors for SI were the presence of poultry on the farm (OR=3.24, 95% CI: 1.52-6.94), the ability of wild birds to enter the piggery (OR=2.50, 95% CI: 1.01-6.16) and failure to implement effective disinfection measures before workers entered the piggery (OR=2.65, 95% CI: 1.04-6.78).

A KBP study on local pig industry workers comprising 153 pig farmers, 21 pig traders and 16 pig trade workers revealed that only 33.7% of those surveyed believed that SI could infect humans, and many undertook practices that were unsafe for SI. The lack of awareness about the zoonotic risk of SI (OR = 3.19, 95%CI: 1.67 - 6.21) was associated with not using personal protective equipment when having contact with pigs.

Social network analysis on the movement of live pigs through four local wholesale live pig markets indicated that the source counties with the highest risk of having SI via the market trading system were in the central, northern and western regions of Guangdong Province. Risk-based control strategies were shown to result in a greater reduction of the magnitude of a potential epidemic of SI compared to a non-targeted control strategy.

Analysis of three year’s sero-surveillance data on SI highlighted that pig farms from south China had exposure to multiple strains of influenza A, including human and avian strains. Spatial modelling identified determinants, such as elevation above sea level, chicken density and the human population density, as important predictors for avian and human influenza infection in pigs within counties. The counties in the delta area of the Pearl River in Guangdong Province and those surrounding Poyang Lake in Jiangxi province had a higher risk of infection with avian or human influenza strains in pigs than other counties in Guangdong, Guangxi, Jiangxi and Fujian provinces.

It is concluded that SI is endemic in south China and, although there is the potential for the emergence of pandemic strains of porcine origin, improved on-farm biosecurity and changes to husbandry and trade practices could minimise the likelihood of a pandemic occurring.

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Abbreviations

ADG	Average Daily Growth
ASF	African Swine Fever
CAHEC	China Animal Health and Epidemiology Center
cDNA	Complementary DNA
CI	Confidence Interval
ECEs	Embryonated Chicken Eggs
ELISA	Enzyme-Linked Immunosorbent Assay
FCE	Feed Conversion Efficiency
GWCC	Giant Weakly Connected Component
HA	Hemagglutinin
HI test	Hemagglutination Inhibition Test
HPAI	Highly Pathogenic Avian Influenza
KBP	Knowledge, Beliefs and Practices
MDA	Maternally Derived Antibody
MDCK	The Madin-Darby Canine Kidney
Mhp	*Mycoplasma hyopneumoniae*
NA	Neuraminidase
NP	Nucleoprotein
OR	Odds Ratio
RT-PCR	Reverse Transcription-Polymerase Chain Reaction Test
RT-qPCR	Quantitative Reverse Transcription PCR
S/N ratios	Sample-To-Negative Ratios
SI	Swine Influenza

SIV	Swine Influenza Virus
SNA	Social Network Analysis
UK	The United Kingdom
USA	The United States Of America
VI	Virus Isolation

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CHAPTER 1: Introduction

1.1 Swine influenza

Swine influenza (SI) is a respiratory disease of swine caused by an influenza A virus that can result in significant economic loss to the pig industry (Kothalawala, Toussaint et al. 2006). Swine influenza is highly contagious and is one of the most prevalent diseases circulating within the global pig population (Choi, Goyal et al. 2002, Liu, Wei et al. 2011, Corzo, Culhane et al. 2013, Kyriakis, Rose et al. 2013). In endemic areas over 90% of herds can be affected (Corzo, Culhane et al. 2013), with over 60% of animals being seropositive within infected herds (Er, Skjerve et al. 2016).

Based on serologic performances of the hemagglutinin (HA) and neuraminidase (NA) proteins, influenza A virus is further subdivided into 18 HA subtypes and 11 NA subtypes (Wu, Wu et al. 2014, David, David et al. 2020). In each HA subtype, there are different strains which may have different pathogenicities (Yang, Chen et al. 2015). The dominant influenza subtypes that are currently circulating in pigs are H1 and H3 (Brown 2000). However, bidirectional cross-species transmission of virus between pigs and birds or humans can occur occasionally (Grontvedt, Er et al. 2013, Nelson and Vincent 2015). Thus, swine influenza is also potentially a threat to public health. The influenza virus genome is segmented, and reassortment happens when two strains replicate within a single cell (Hause, Collin et al. 2014). The coexistence of different swine influenza virus (SIV) strains on a farm can result in the production of new strains with potentially diverse pathogenicities and even zoonotic capacities.

Spill-over infections of SIV to humans have been observed since 1918 (Bui, Chughtai et al. 2017). A triple-reassortant swine H1N1 influenza virus infected both people and pigs in an outbreak in 2007 in the United States of America (USA) (Killian, Swenson et al. 2013) and in China. Human cases resulting from infection with swine influenza H3N2 virus and European avian-like swine H1N1 influenza virus were also reported in 1999 and 2010, respectively (Gregory, Lim et al. 2001). Exposure to live pigs is the main reason for humans becoming infected with SIV (Lopez-Robles, Montalvo-Corral et al. 2012) and understanding the zoonotic risk of influenza A at the pig-human interface is vital for developing strategies to mitigate against the risk of zoonotic SIV infection in human.

1.2 Pig industry in China

China has the largest pig population in the world with 447.2 million pigs raised in 2018, representing 31.4% of the world's pig population at that time (Food and Agriculture Organization of the United Nations 2020). Subsequently this percentage has reduced due to an outbreak of African swine fever (ASF) which commenced in August 2018. At the end of 2017, the total number of Chinese pig farms was 37.8 million, and there were 433.2 million pigs, including 43.93 million sows, in China (Ministry of Agriculture and Rural Affairs 2018).

Small scale farms are reportedly the dominant pig production system in China with more than 90% of pig farms having an annual output of less than 50 head in 2017 – contributing approximately 25% of the annual pigs sold (Ministry of Agriculture and Rural Affairs 2018). In contrast, farms that sold more than 500 head in 2017 contributed about 47% of the total pigs sold in that year (Figure 1.1).

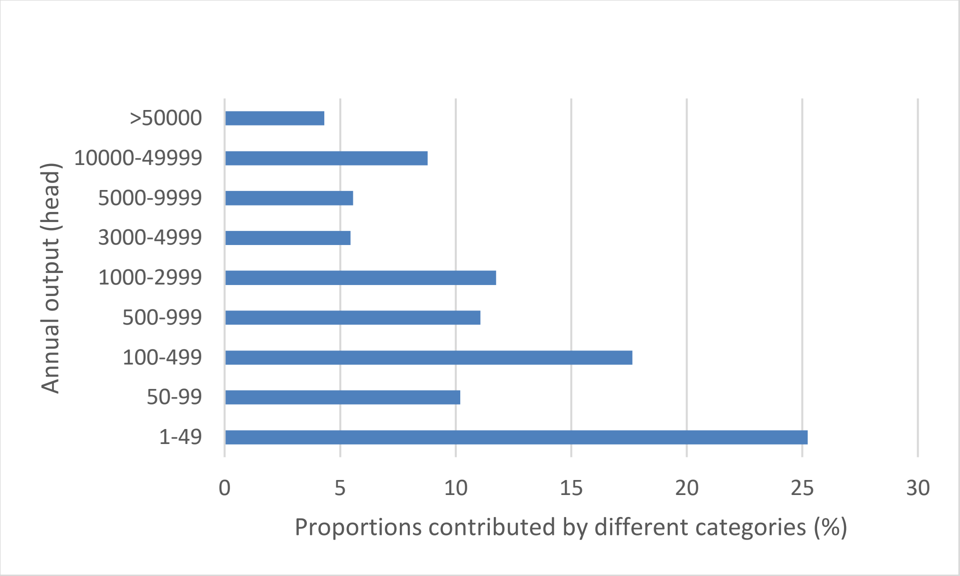

Figure 1.1 Contributions of pig farms of different size to the total pig population in China in 2017 (Data sourced from Chinese livestock statistic book 2018, Ministry of agriculture and rural affairs (2018)).

The pig population is not geographically evenly distributed in China. The provinces with the highest pig density are located in the central, eastern and southern regions of China, including the provinces of Henan, Shandong, Jiangsu, Hunan and Chongqing (Figure 1.2).

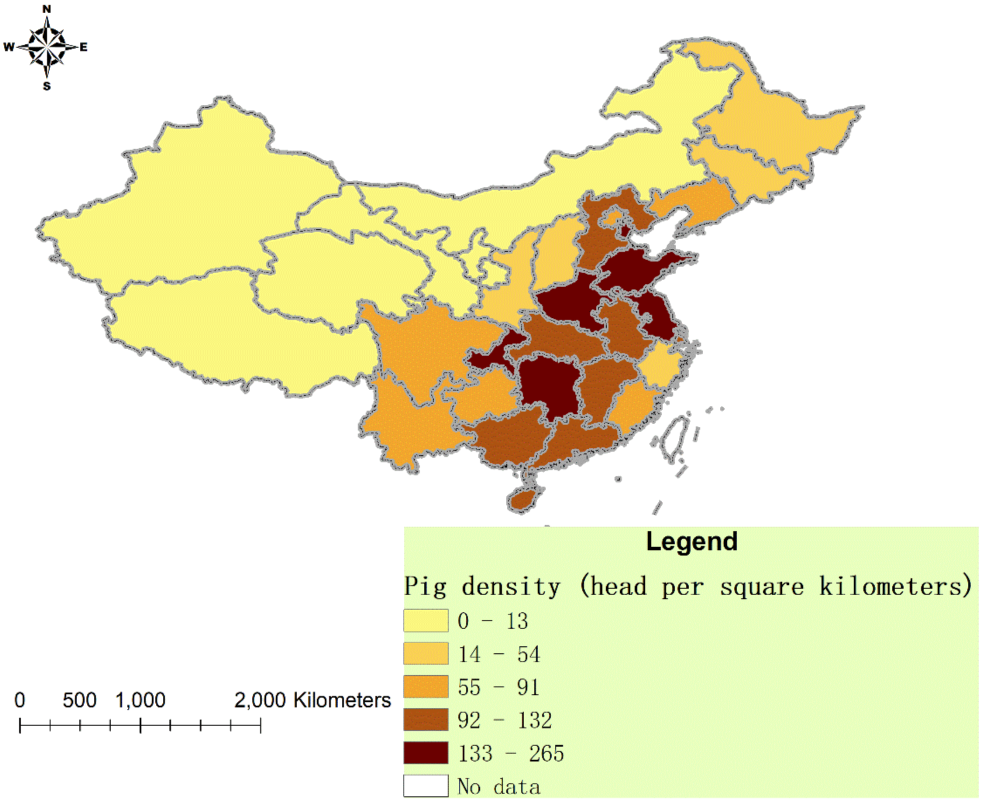

Figure 1.2 Pig density in different provinces in China in 2017 (Data sourced from Chinese livestock statistic book 2018, Ministry of agriculture and rural affairs (2018)).

1.3 Swine influenza in China

Swine influenza is prevalent in the Chinese pig population (Chen, Zhang et al. 2013). Several subtypes of influenza virus, including H1, H3, H4, H5, and H9, have been detected in the Chinese pig population (Ninomiya, Takada et al. 2002, Yu, Zhou et al. 2011).

South China has long been thought of as "an epicentre of influenza", because of the unique ecosystem containing vast wetlands, presence of live animal markets, and the contact between humans, pigs and poultry in this area. Some studies have offered evidence that SI is not evenly distributed throughout China and is more prevalent in south China than north China (Yu, Zhou et al. 2011).

There is a unique live pig market trading system in Guangdong Province, south China, and this market trading system may play a role in the spread of SIV among local farms. It has previously been observed that traders in livestock markets have a higher risk of being infected with SIVs (Ma, Anderson et al. 2015). However, previous studies have failed to address the human-pig contacts at the human-pig interface in Chinese live pig markets. Furthermore, contacts between poultry, pigs and pig industry workers potentially play a significant impact on the cross-species infection of influenza (Saenz, Hethcote et al. 2006, Gray and Kayali 2009, Dorjee, Revie et al. 2016). Therefore, conducting systematic surveillance on SIV in south China is not only valuable for potentially preventing new SI epidemics in the local pig industry, but is also critical for preparing for potential pandemic human influenza from swine-sourced influenza.

1.4 Research Aims

Studies on SI in China have mainly focused on the phylogenetic analysis of isolates with pig level prevalence summarized only from the results of passive surveillance data (Chen, Zhang et al. 2013, Li, Fu et al. 2015). However, currently SI is not a notifiable animal disease in China, and surveillance of SI in China hasn't provided adequate information on the epidemiological characteristics of the occurrence, distribution and spread of the disease. Both the herd prevalence of SIV infection in China and the biosecurity practices used on Chinese pig farms haven't been studied in detail in previous studies. For the purpose of controlling SI in China and preparing for a potential pandemic of human influenza from swine-sourced influenza, risk-based surveillance is required. Consequently the aims of the research included in this thesis were to:

Describe the husbandry and biosecurity practices in pig farms in Guangdong Province, south China;

Evaluate the farm-level prevalence of SIV infection and identify relevant risk factors for SIV infection in pig farms in Guangdong Province;

Describe the knowledge, beliefs and practices of pig industry workers on SI to evaluate the risk of zoonotic transmission of SIV at the human-pig interface in Guangdong Province;

Describe the network characteristics of the live pig market trading system in Guangdong Province and identify the key source counties with higher connectivity in this trading network;

Provide recommendations for risk-based disease control strategies in south China, based on the findings of the live pig market trading network.

Describe multi-strain swine influenza coinfection and avian/human influenza infection in pigs and determine the environmental factors associated with these infection scenarios.

1.5 The layout and format of this thesis

The overall aim of this thesis was to inform control of SI in south China, especially in Guangdong Province. The thesis comprises a series of studies that address different aspects of the epidemiology of SI in south China. All the results chapters (3 to 6) have already been published (3, 4 and 5) or have been submitted to and are presently under review (6) in international peer-reviewed journals. These result chapters contain the same text and structure as published or submitted to the respective journals. The formatting and referencing have been altered to form a standard style for all chapters. In the final chapter, a summary and discussion of all findings are presented.

The literature on swine influenza is reviewed in Chapter Two. The global distribution, diagnosis, risk factors, zoonotic mechanism of SI and gaps in the control of SI in China are introduced. Specific methods used in this thesis (social network analysis and machine learning) are also briefly outlined in this chapter.

In Chapter Three, the results of a cross-sectional survey conducted to evaluate pig farmers' perceived herd prevalence of swine influenza in Guangdong Province are presented. Risk factors for a farm being a case farm were analysed using logistic regression analysis. The routine husbandry and biosecurity practices adopted by pig farms in Guangdong Province are also described in this chapter.

In Chapter Four, the results of a KBP (knowledge, beliefs and practices) study employed to describe the knowledge, beliefs and practices of pig industry workers on SI to evaluate the risk of zoonotic transmission of SI at the human-pig interface in Guangdong Province are presented. The risk factors for the pig industry workers' low awareness of the zoonotic potential of SI and "not using personal protection equipment when contacting pigs in work" were investigated in detail.

In Chapter Five, the results of social network analysis (SNA) undertaken to describe the live pig market trading network in south China are presented. The movement data in all of the wholesale pig markets in Guangdong Province were analysed to explore the structure of the network and to identify the source counties that had higher connectivities. A model was developed to illustrate the benefit of implementing a risk-based intervention in terms of decreasing the magnitude of a potential epidemic.

In Chapters Six, the results of comprehensive passive surveillance data of SI analysed to describe human and avian source influenza infection in pigs in south China are summarised. Anthropogenic, meteorological and geographical factors associated with SI infection scenarios were explored. Predicted risk maps were generated to inform future targeting surveillance on SI.

Finally, in Chapter Seven, how the findings of the thesis would benefit SI control in south China and the limitations of the current study are discussed and summarised. Further studies were also proposed in this chapter.

CHAPTER 2: Literature Review

2.1 Swine influenza

Swine influenza is a respiratory disease of pigs caused by influenza A viruses. The typical clinical signs associated with the disease include coughing, laboured breathing, nasal discharge, sneezing and pyrexia (Kothalawala, Toussaint et al. 2006) and lesions of pneumonia may be observed in infected pigs at slaughter (Karasin, Brown et al. 2000, Vincent, Lager et al. 2008, Rose, Herve et al. 2013). Reproductive problems, including abortion and stillbirths, have also been reported in sows infected with SIV (Wesley 2004). Subclinical infection is common, especially in herds with antibody against homologous SIV strains (Choi, Goyal et al. 2004, Rose, Herve et al. 2013, Hemmink, Morgan et al. 2016).

Influenza A viruses belong to the Orthomyxoviridae family, which are enveloped viruses with eight single strand RNA segments. Subtypes of influenza A viruses are determined by the antigenic and genetic properties of the two major surface proteins, hemagglutinin (HA) and neuraminidase (NA) (Hause, Collin et al. 2014). Currently, there are 18 HAs (H1-H18) and 11 NAs (N1-N11) recognised, with the H17-18 and N10-11 types having only recently been isolated from bats (Mehle 2014).

Influenza A viruses are the most clinical important influenza viruses as they can cause serious disease in a wide range of species, including humans, pigs, birds, horses, cattle, whales, seals, tigers, dogs, cats and ferrets (Mehle 2014). There are three other genera of influenza viruses: Influenza B, C and D. Influenza B viruses have mainly been isolated from humans and seals (Osterhaus, Rimmelzwaan et al. 2000). Influenza C viruses are primarily found in humans, pigs and dogs, and influenza D has recently

(2011) been detected in pigs and cattle (Hause, Collin et al. 2014, Luo, Ferguson et al. 2017).

Swine influenza was first observed in 1918 in the USA, Hungary and China (Brown 2000) and today it is one of the most ubiquitous diseases circulating in the global pig population. Choi, Goyal et al. (2002) reported that 22.8% of individual pigs were seropositive in the USA using the hemagglutination inhibition (HI) test, while Corzo, Culhane et al. (2013) reported a much lower individual prevalence level (4.6%), although they did report a 90.6% herd prevalence using a real-time reverse transcription polymerase chain test (real-time RT-PCR). The difference in the individual animal level prevalence between studies could be due to the different sampling strategies adopted. The first study summarized data from a veterinary diagnostic laboratory, while the latter one used a random sampling approach. These two studies also used different tests, and the samples were collected from different regions in different years, all potentially influencing the findings. A cross-sectional study in northern Mexico reported that more than 50% of the samples from commercial farms (300-2500 sows) were seropositive to either H1 or H3 subtype SIV. However, the seroprevalence may have been overestimated because these authors primarily sampled pigs less than ten weeks of age and maternally derived antibody (MDA) can last up to ten weeks in pigs and potentially have resulted in false positive results (Cador, Herve et al. 2016). This assumption was also supported by the finding of decreasing antibody titres with the age of the sampled pigs. The authors also reported that 16.7% (25/150) of the sampled pigs were positive for type A influenza with a RT-PCR test (Lopez-Robles, Montalvo-Corral et al. 2014).

Swine influenza is also widespread in Europe. In a study in Belgium, France, Italy and Spain, 80 farrow-to-finish farms were monitored from 2006 to 2008. Ninety percent of the farms were classified as positive for SIV, with an individual level seroprevalence of 62%. Forty-nine percent of the farms were infected with one subtype, 38% with two subtypes and 3.9% with three subtypes of SIVs (Kyriakis, Rose et al. 2013). However in this study the sampling was also biased, resulting in potential overestimation of prevalence as farms sampled were selected from areas with a high density of pigs or contained pigs that had a history of respiratory problems. An analysis of historical surveillance data in Norway showed that the national herd seroprevalence of influenza A(H1N1)pdm09 virus, which is an influenza A virus strain circulating in both humans and pigs, was around 43%, and the individual pig prevalence of pandemic H1N1 in infected farms was more than 60% (Er, Skjerve et al. 2016). Another study in Spain in 2009 that sampled pigs from 98 randomly selected pig farms, reported a farm-level seroprevalence of nearly 100% with an animal level seroprevalence of 62.3% (Simon-Grife, Martin-Valls et al. 2011). In England, a 52% herd prevalence was reported by Mastin, Alarcon et al. (2011), with the highest individual prevalence of 33% being reported in sows.

Swine influenza is endemic in the Chinese pig population, with many subtypes contemporarily circulating on farms. Serological evidence indicates the presence of H1, H3, H4, H5, H7 and H9 influenza viruses in pig populations in the country (Ninomiya, Takada et al. 2002, Liu, Wei et al. 2011, Yu, Zhou et al. 2011). Liu, Wei et al. (2011) reviewed the data from 10 years of publications and concluded that the average individual seroprevalence to subtypes H1, H3, H5, H7 and H9 were 31.1, 28.6, 1.3, 0 and 2.4%, respectively. Song, Xiao et al. (2010) reported an individual pig prevalence of more than 50% for H1 and H3 in commercial farms in Fujian province. No H5N1 infection was detected in pigs in that study, and while infection with H9 was detected, it was only at a low seroprevalence (1% in 2004 and 2.6% in 2007). Liu, Zhou et al. (2014) reported 52% of pigs were positive for H1N1, and 16.9% were positive for H3N2 in Tibet.

Studies on SI in China have mainly focused on the phylogenetic analysis of isolates with individual prevalence summarized only from the results of passive surveillance data. The herd prevalence of SIV infection in China has previously rarely been reported, and it is likely that the reported individual animal prevalences are biased through the sampling methodology adopted.

Pigs can be infected with more than one subtype of SIV, for example, two case studies in China reported that 8.8 and 24% of the tested pigs were positive for both H1 and H3, respectively (Song, Xiao et al. 2010, Liu, Zhou et al. 2014). Choi, Goyal et al. (2002) also reported that 7 out of 480 samples of pig lungs tested at a veterinary diagnostic laboratory in the USA contained both H1N1 and H3N2 viruses using RT-PCR and sequencing. A study in Spain also reported that mixed infections of H1N1, H1N2 and H3N2 were detected in 60% of the sampled farms (Simon-Grife, Martin-Valls et al. 2011). Takemae, Shobugawa et al. (2016) reported that a pig farm in Thailand with more than 1000 pigs was more likely to have reassortant SIVs infections. The authors speculated that larger herds were more likely to have multi-strain infections compared with smaller herds due to greater opportunities for different circulating SIV.

Pigs are believed to act as a "mixing vessel" for swine, avian and human influenza viruses allowing the production of reassortant influenza viruses. Most avian influenza viruses only bind via an α-2, 3-galactose sialic acid linkage, which is abundant in the epithelial cells of the avian trachea, while most human influenza viruses prefer α-2, 6-galactose sialic acid linkage, which is abundant in epithelial cells of the human trachea. However the epithelial cells of the pig trachea have both α-2, 3- and α-2, 6-galactose sialic acid linkages, and so pigs can be susceptible to both avian and human influenza viruses (Ito, Couceiro et al. 1998).

Despite the potential impact of SIV on public health, SI is often neglected by the pig industry and also by researchers. The main reason for this is that many of the infections in pigs are subclinical or mild and hence easily overlooked (Detmer, Gramer et al. 2013). Furthermore although the morbidity of SIV infection in a herd can be as high as 100%, mortality is usually extremely low (Er, Lium et al. 2014).

2.2 Swine Influenza Viruses

2.2.1 The characteristics of swine influenza viruses

As a member of the influenza A virus group, SIV is an enveloped virus with eight segments of RNA (HA, NA, PA, PB1, PB2, NP, M and NS) (Reeth, Brown et al. 2012). The infectivity of the virus is mainly determined by the two surface proteins, HA and NA, because these are the proteins that bind receptors in the host and facilitate virus invasion into the host cell (Hause, Collin et al. 2014).

Influenza viruses are sensitive to environmental conditions. Chemical disinfectants such as 0.1 mol/L NaOH, 70% ethanol, 70% 1-propanol and ethylene oxide can effectively inactivate the virus (Jeong, Bae et al. 2010). One study reported that even a powdered laundry detergent with peroxygen (bleach) was sufficient to kill the virus (Lombardi, Ladman et al. 2008). However, it is worth noting that the inactivating efficiency of many disinfectants is reduced at low temperatures and in environments contaminated with organic material, and consequently caution is needed when disinfecting SIV-infected premises during winter and organic matter should be removed prior to disinfection (Botner and Belsham 2012).

Influenza A virus can remain stable and infectious for months under natural conditions, particularly at low temperatures and in the existence of organic matter (Haas, Ahl et al. 1995). One study reported that in slurry at 5°C, the infectivity of SIV was retained for more than six weeks, whilst at 20 °C the virus still remained viable for approximately 14 days (Botner and Belsham 2012).

2.2.2 Subtypes and genetic recombination among strains

Many subtypes of influenza A virus have been isolated from pigs all over the world. Among these subtypes, the most common SIVs circulating in pig populations are subtypes H1N1, H3N2 and H1N2. The dominant strains in the USA are classic H1N1, triple reassortant H3N2 and pandemic A/H1N1 2009 (H1N1pdm09) virus (Bowman, Workman et al. 2014). In Europe, the dominating strains are avian-like swine H1N1, human-like reassortant swine H1N2, human-like reassortant swine H3N2, and H1N1pdm09 virus (Simon, Larsen et al. 2014). In China, all of the lineages from both the USA and Europe are circulating in the pig population (Chen, Fu et al. 2014, Xie, Zhang et al. 2014, Yang, Chen et al. 2016).

Co-circulation of different SIV strains is commonly seen in pig farms. Active surveillance undertaken in the USA reported simultaneous infection with influenza H3N2 and H1N1pdm09 virus in 8 different age categories of pigs from four to over 24 weeks of age (Corzo, Culhane et al. 2013). Another study in Italy reported infection with multiple reassortant genotypes of H1N2 in one local commercial breeding farm during the two-month study period (Beato, Tassoni et al. 2016). It has also been reported that 24% of the tested pigs in South China were positive for both H1 and H3, and such cases were discovered in seven out of the nine counties surveyed (Song, Xiao et al. 2010).

The coexistence of different SIV strains facilitates frequent gene reassortment on pig farms. A cohort study in three selected farrow-to-finish pig farms in France found that H1N1 and H1N2 viruses could simultaneously exist in the same farm, batch or even individual pigs, and reassortants between viruses from these lineages could be isolated from infected herds (Rose, Herve et al. 2013). After the pandemic of "swine flu" in 2009, gene reassortment between H1N1pdm09 viruses and local endemic swine viruses were identified in many countries, including the USA, Brazil, Germany, Italy, UK, Vietnam, Thailand, Japan, Korea and China (Abe, Mine et al. 2015, Kong, Wang et al. 2015). Whole-genome phylogenetic analysis of 368 influenza A viruses circulating in the USA demonstrated the presence of 44 different genotypes of H3N2 in that country from 2009 to 2016, with the majority of these genotypes containing at least one gene segment from H1N1pdm09 (Rajao, Walia et al. 2017).

Swine influenza virus reassortants can become endemic in pig farms and potentially transmit to humans resulting in pandemic circulation. The best-known example is the 2009 H1N1 pandemic influenza A virus which is a reassortment of three different influenza strains circulating in pigs, birds and humans (van der Meer, Orsel et al. 2010). Shortly after the outbreak of H1N1 in the USA, the H1N1pdm09 virus was found in the pig and human population all over the world. A survey undertaken in North Vietnam in 2009 reported a maximum seroprevalence of H1N1pdm09 of 55.6% (95% CI: 38.1-72.1) in pigs sampled at a slaughterhouse, with a farm-level seroprevalence of 29% (95% CI: 23.2-35.7) (Trevennec, Leger et al. 2012). In China, the H1N1pdm09 virus was also first isolated in pigs in 2009, and reassortants with internal genes from the pandemic 2009/H1N1 viruses were found in pigs in the following years (Chen, Zhang et al. 2013, Chen, Zhang et al. 2014, Qiao, Liu et al. 2014). H3N2 variants containing genes from the H1N1pdm09 influenza virus were subsequently isolated from at least seven countries between 2009 and 2013, and H3N2 seems to be the most commonly emerging SIV subtype (Kong, Wang et al. 2015). It was suspected that the H1N1pdm09-origin internal gene segments appeared to have an advantage over the segments of other SIV's in terms of contributing genes for new reassortants (Kong, Wang et al. 2015).

Although gene reassortants and variants of H1N1pdm09 and other SIVs have been reported in China, the prevalence, geographical and population distribution, and relevant risk factors for their occurrence are still not clear.

2.3 Diagnosis of swine influenza

2.3.1 Serological methods

Serological tests for SIV mainly refer to tests targeting host antibodies against the virus. The most commonly used serological tests are the hemagglutination inhibition (HI) test and the enzyme-linked immunosorbent assay (ELISA). Many commercial ELISA kits have been developed to detect antibody to the influenza A nucleoprotein (NP) because NP is highly conserved in influenza A viruses (Goodell, Prickett et al. 2016). Several studies have shown that an NP blocking ELISA kit for testing antibodies in birds can also be used to detect NP antibodies in pigs (Nava, Merino et al. 2013, Goodell, Prickett et al. 2016). In general, HI tests are simpler to operate, cheaper and quicker than ELISAs; however only one subtype can be identified with each HI test. In addition, the sensitivity of the HI test can be low if used solely for

SIV surveillance when heterologous viruses are present (Goodell, Prickett et al. 2016), although they do offer the advantage that they can be used for subtyping the viruses (Van Reeth, Labarque et al. 2006).

Serological surveys/tests often take advantage of existing collections of serum samples, as collecting blood samples involves significant cost, time and labour inputs (De Lucia, Rambaldi et al. 2019). To overcome the issues with collecting blood samples, a new method targeting the antibody in the oral fluid of swine, using a NP-blocking ELISA, has been developed (Panyasing, Goodell et al. 2014). With experimentally infected pigs, the NP antibodies in the oral fluid were detected 7 to 42 days post-infection in all challenged groups. The oral fluid versus serum sample-to-negative (S/N) ratios from pigs in the same pen showed a correlation of 0.796, indicating good agreement between results for testing oral fluid samples compared with serum samples (Panyasing, Goodell et al. 2014). In contrast, another study that used field-collected oral samples found that the NP blocking ELISA had a much lower sensitivity in 10–14-week-old pigs compared with matched serum samples (19% for oral fluid and 93% for serum - $P < 0.01$) (Priscilla, Lorna et al. 2017).

There are several advantages with using serological tests: firstly, they are often inexpensive; secondly, they are easier to perform compared with PCR tests or virus isolation; and thirdly, serological tests are more sensitive in detecting exposure of pigs to influenza A virus than PCR tests or virus isolation because the antibodies can last for at least 1.5 months post-infection, and consequently, serological tests are less sensitive to the sampling time (Goodell, Prickett et al. 2016, Priscilla, Lorna et al. 2017). However serological tests have limitations which include: they only provide information on historical exposure to SIV and do not provide viral genetic information or the live viruses which are vital for evaluating the potential pandemic threat of strains; cross-reactions can occur between different lineages within one subtype, or even among different subtypes; and maternally derived antibodies may interfere with the accuracy of the test (Allerson, Deen et al. 2013, Detmer, Gramer et al. 2013).

2.3.2 Polymerase chain reaction methods

Polymerase chain reaction (PCR) tests are mainly used in surveillance to detect the presence of SIV genes and to produce amplicons for further gene sequencing. The conventional RT-PCR targets the M gene of influenza virus and amplifies cDNA from the viral RNA. For subtyping, specific primers need to be designed to detect different subtypes. Universal primers can also be used to amplify cDNA, which is then sequenced (Inoue, Wang et al. 2010).

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) assays for the detection of SIVs were developed in 2004 (Richt, Lager et al. 2004). Compared with conventional RT-PCR, the real-time RT-PCR assay can be performed in a shorter time (within a few hours) and can differentiate SIV subtypes. It can also be less expensive than virus isolation (VI) and conventional RT-PCR assays. Most importantly, real-time RT-PCR doesn't require post–PCR sample handling, thus reducing the potential for cross-contamination (Richt, Lager et al. 2004).

For public health purposes, detection of coinfection with different virus strains in a pig herd would be very valuable in SIV surveillance. Multiplex RT-qPCR assays can differentiate the H1, H3, N1 and N2 SIV subtypes. These multiplex RT-qPCR assays can also identify different lineages within the H1 subtype, such as "av" (European avian-derived), "hu" (European human-derived) and "pdm" (H1N1pdm09). Henritzi, Zhao et al. (2016) reported that the multiplex RT-qPCR assays that they developed could detect double infections with different lineages in one clinical sample. However, the efficiency of the RT-qPCR relies heavily upon the specific primers used, with outdated primers resulting in low test sensitivity (Yang, Kuo et al. 2014). Since the primers used by Henritzi, Zhao et al. (2016) were designed specifically for the SIV strains circulating in Europe, whether these RT-qPCR assays could be used in SIV surveillance in other regions/continents requires further study.

2.3.3 Virus isolation

Isolation of SIV is undertaken routinely in embryonated chicken eggs (ECEs) and various cell lines, including the Madin-Darby canine kidney (MDCK) and the CACO-2 cell line (Chiapponi, Zanni et al. 2010). It has been reported that the sensitivity of SIV isolation with different methods is dependent upon the virus strains present. A study using strain A/Swine/Indiana/1726/88 (H1N1) showed that ECE was more sensitive than an MDCK cell line (Clavijo, Tresnan et al. 2002). In contrast in another study with clinical samples, use of the MDCK cell line resulted in recovery of more isolates of H1N2 and H3N2 than with ECE (Bowman, Nelson et al. 2013), whilst the CACO-2 line was shown to be more sensitive (Fisher's exact test, $p<0.01$) for the isolation of H1N1 and H1N2 subtypes in Italy compared to both MDCK cells and ECEs (Chiapponi, Zanni et al. 2010). However for influenza A H3N2 virus, isolation in ECE has been demonstrated to be better than in cultured cells ($p<0.01$) (Chiapponi, Zanni et al. 2010).

Virus isolation is often difficult, expensive and time consuming, but it is necessary when the live virus is required for further research, such as evaluating the pathogenicity of new SIVs and screening for vaccine candidate strains (Detmer, Gramer et al. 2013).

2.4 Epidemiology

Swine influenza is endemic in many countries in North and South America, Europe, Asia and Africa (Almeida, Storino et al. 2017). Infection in pig farms can be seen throughout the year, although an increased number of cases are often seen in spring and winter (Beaudoin, Johnson et al. 2012, Kyriakis, Rose et al. 2013). It is believed that commercial pig farms have a higher risk of infection compared to backyard farms, especially for infection with new SIV reassortants (Gonzalez-Reiche, Ramirez et al. 2017). Farrow-to-finish pig farms are more susceptible to SIV infection than fattener enterprises because they continuously produce naïve piglets (Loeffen, Nodelijk et al. 2003, Kyriakis, Rose et al. 2013). In an infected herd, sows have the highest risk of being seropositive, most likely linked to their older age resulting in greater opportunity for exposure to the virus, while the greatest chance of isolating live viruses is from piglets (Mastin, Alarcon et al. 2011, Takemae, Parchariyanon et al. 2011, Ozawa, Matsuu et al. 2015, Er, Skjerve et al. 2016).

Virus transmission between pigs is mainly through direct pig-to-pig contact. Aerosol transmission is one of the common ways of indirect transmission of SIV (Brown 2000, Corzo, Romagosa et al. 2013, Hemmink, Morgan et al. 2016). A pig farm can become infected through the introduction of carrier pigs or entry of the virus on contaminated visitors, vehicles or fomites (Simon-Grife, Martin-Valls et al. 2011, Allerson, Cardona et al. 2013, Er, Skjerve et al. 2016).

In infected pigs SIV is excreted in oral and nasal secretions, with no virus shed in the faeces (Choi, Goyal et al. 2004, Botner and Belsham 2012). Pigs can start to shed virus within 2 days of infection. Although the duration of shedding is usually 8 to 10 days, shedding for more than 30 days has been reported (Choi, Goyal et al. 2004, Botner and Belsham 2012). The reason for a long shedding period has been postulated to be linked to the suppression of immunity in infected pigs (Choi, Goyal et al. 2004). Between individual pigs within a herd, the transmission of SIV can be rapid. Rose, Herve et al. (2013) reported that in farrow-to-finish pig farms with recurrent influenza outbreaks the basic reproduction value (R0) was high, between 2.5 and 6.9.

2.5 Interspecies transmission

The HA subtypes circulating between birds, pigs and humans include H1-H16, with different subtypes predominantly circulating in individual species. Wild waterfowl are the natural reservoir of H1-H16, while domestic chickens are mainly infected by H5, H7 and H9 subtypes. For humans and pigs the most common circulating subtypes are H1-H3 and H1 and H3, respectively (Short, Richard et al. 2015). Pigs can contract influenza A viruses from other species, especially from infected humans and birds (Karasin, Brown et al. 2000, Grontvedt, Er et al. 2013, Nelson and Vincent 2015).

Avian influenza viruses have been isolated from pigs in many countries and regions. In Canada, H4N6 influenza A isolates were isolated from pigs with pneumonia on a commercial swine farm and similarly an avian-origin H4N6 was isolated from pigs displaying clinical respiratory signs in the USA in 2015 (Karasin, Brown et al. 2000, Abente, Gauger et al. 2017). A study in Nigeria reported that 22 of 129 samples collected from apparently healthy pigs were positive to H5N1 while there was an epidemic of highly pathogenic avian influenza (HPAI) H5N1 in local poultry (Meseko, Globig et al. 2018). In addition, both avian H9N2 and H5N1 viruses were detected in pigs in Egypt in 2014 and 2015 (Gomaa, Kandeil et al. 2018). In China, 28 isolates of H9N2 were detected in pigs from 1998 to 2007 (Yu, Zhou et al. 2011). The isolates of H9N2 AIVs recently detected circulating in poultry farms in south China have shown increased ability to replicate in pigs than did earlier isolates (Sun, Lin et al. 2019), highlighting the greater risk of new viral reassortants appearing in this location.

In pigs, infection with human source influenza A appears to be more common than that from avian-sourced influenza A (Nelson and Vincent 2015). Introductions of human seasonal influenza viruses into pigs during the period from 1965 to 2013 has been summarized by Nelson, Stratton et al. (2015), and the authors concluded that more than 40 cases of human-origin H1N1 viruses in pigs had been reported in the 5 years after H1N1pdm09 was initially detected in humans. A study in the Czech Republic reported the presence of antibodies against the human influenza virus isolated during the 1995 epidemic in the local pig population. It is possible that the human virus was introduced to the pig herds by infected animal attendants, in whom antibodies against this virus were also found (Pospisil, Lany et al. 2001). H3N2 viruses closely related to human viruses that circulated in 2010 have also been found in pigs from Central America in 2010 (Gonzalez-Reiche, Ramirez et al. 2017). In China, former prevailing human H1N1 strains have also been found to be circulating within the pig population (Yu, Zhou et al. 2009) and phylogenetic analysis indicated that these viruses arose through transmission from humans to pigs. It was interesting that in that study 4 out of 5 virus isolates were from Guangdong province. This may be either because Guangdong actually had more pig infections arising from human influenza than other provinces, or that Guangdong had contributed more of the 500 tested samples. Although the samples were sourced from 8 different provinces, unfortunately the actual sample size from each province was not given (Yu, Zhang et al. 2007).

Many researchers believe that most subtypes of influenza A viruses from other species are capable of transiently infecting pigs. However as the majority of these strains have not been repeatedly detected in the same pig farms or in samples collected from pigs processed at slaughterhouses it is assumed that they are not able to establish in the pig population (Pospisil, Lany et al. 2001, Vijaykrishna, Smith et al. 2011, Li, Zhou et al. 2015).

2.6 Risk factors for SIV infection on pig farms

2.6.1 Husbandry factors

Some management and husbandry practices are associated with SIV infection in pig farms. Larger farms have been reported to have an increased risk of infection than smaller herds (Mastin, Alarcon et al. 2011, Takemae, Shobugawa et al. 2016, Gonzalez-Reiche, Ramirez et al. 2017). A high density of weaners has also been shown to increase the risk of infection in herds (OR: 2.9; 95% CI: 1.2–7.0) as has failure to adopt an all-in all-out practice in the fattening room (OR = 2.4, 95% CI: 1.0–5.8) (Fablet, Simon et al. 2013). Another study reported that herds with a high number (>18) of finishers per water space had an increased risk of infection (OR 5.22; 95%CI: 1.57 – 17.43) compared to herds with lower numbers of pigs (≤ 18) per water space (Mastin, Alarcon et al. 2011). Another study also found that the presence of open partitions between pens increased the risk of infection (Simon-Grife, Martin-Valls et al. 2011), most likely associated with increased contact opportunities between pigs. Other factors which have also been linked with an increased risk of SIV infection in pig herds include increased replacement rates in pregnancy units, farm type (farrow-to-finish and breeder herds had a higher risk of SI infection than finisher farms), having a suckling period of less than 28 days (for prevalence in weaners) and a fully slatted floors in pens (Simon-Grife, Martin-Valls et al. 2011, Baudon, Peyre et al. 2017). Low room/ambient temperature (<25°C) in the farrowing room has also been reported to increase the risk of infection (Fablet, Simon et al. 2013). In the United Kingdom, intensively housed (indoors) pigs had a higher risk of SIV infection than farms with extensive or outdoor housing (Mastin, Alarcon et al. 2011). In contrast the use of straw yards in UK farms has been shown to reduce the risk of infection (Mastin, Alarcon et al. 2011, Fablet, Simon et al. 2013).

In conclusion, the husbandry practices that would facilitate interactions between naïve pigs and introduce stress to pigs are potential risk factors for SI infection in pig farms. Although extensive research on the risk factors for SI infection has been carried out in many countries, no single study existed addressing this topic in Chinese pig farms prior to the studies presented in this thesis.

2.6.2 Biosecurity factors

Poor biosecurity nearly always leads to a higher risk of a range of diseases, including SI (Filippitzi, Kruse et al. 2018). At least three biosecurity factors have been reported to be associated with increasing the risk of SIV infection in pig farms. Firstly, frequent human-pig interaction is a factor as human influenza viruses can spillover to pigs. One study demonstrated that the presence of farm staff with influenza-like illness was significantly associated with the presence of SIV on pig farms in Norway (OR = 4.15, 95%CI 1.5–11.4, $p = 0.005$) (Grontvedt, Er et al. 2013). A lower herd-level seroprevalence in Norwegian fattening herds was believed to be associated with fewer close human-pig interactions, in contrast to sow (breeding) herds which had the highest seroprevalence because sows frequently had contact with many different people (Er, Skjerve et al. 2016). Secondly, uncontrolled access to the farm by vehicles or visitors can increase the chance of introducing diseases through contaminated vehicles, clothing, footwear and fomites. Uncontrolled access to farms has been found to be a risk factor for H1N1 seropositivity (OR = 2.44, 95% CI: 1.01–5.87) in a study conducted in Spain (Simon-Grife, Martin-Valls et al. 2011). A third factor is disease management within the farms. Mastin, Alarcon et al. (2011) reported that the management of the sick pen was important; stating that the location of sick pens in a separate building to those housing healthy pigs may help reduce SIV infection, although this was not confirmed through a formal study. However, as with most infectious diseases, isolation of affected animals is a key management procedure to minimise transmission to other animals and contamination of the environment (Cui and Chen 2017).

Most of the studies on risk factors for infection with SI have found agreement in risk and protective factors, although some studies did generate conflicting results. For example, Simon-Grife, Martin-Valls et al. (2011) reported that the presence of other species, such as cats, dogs, birds or cattle, on the farm increased the infection risk, in contrast Takemae, Shobugawa et al. (2016) found that the presence of other animals on the farm was potentially protective. These conflicting results may be due to the different ecosystems and the different husbandry practices adopted in the surveyed herds, different populations under study or differences in the case definitions in the individual studies.

2.6.3 Environmental factors

Environmental factors for SIV infection have rarely been studied; however, the density of pig farms in an area appears to be a risk factor for SIV infection. Pasma (2008) analysed H3N2 SI outbreaks in Canada during the autumn of 2004 and found clustering of outbreaks in a region with a high pig density. It was hypothesized that the density of pig farms was a factor in the clustering and spread of this outbreak, although the data didn't show statistical significance for this factor. Couacy-Hymann, Kouakou et al. (2012) also thought the low pig density in Côte d'Ivoire, Benin, and Togo might be the reason for the low prevalence of avian and swine influenza in those three African countries.

Some studies on avian influenza have highlighted the role of environmental and meteorological factors in avian influenza outbreaks. Potential risk factors, such as monthly average rainfall in the preceding 3-7 months, being close to rivers, lakes or seacoasts, low ambient air temperature, and high relative humidity have been reported to be linked with avian influenza outbreaks (Fang, Cao et al. 2005, Si, de Boer et al. 2013, Zhang, Liu et al. 2014, Ferenczi, Beckmann et al. 2016). Since pigs may also contract avian-source influenza viruses, these environmental and meteorological factors could also be potentially associated with outbreaks of SI and require further investigation.

2.7 Impact of swine influenza on the pig industry

2.7.1 Morbidity and mortality

Swine influenza is a highly contagious disease with almost 100% of exposed pigs becoming infected, although the mortality rate is usually very low. Even with infection in a naïve pig population, clinical signs may only be observed in a small proportion of pigs with Er, Lium et al. (2014) reporting that less than 7% of pigs displayed clinical signs in an outbreak in a boar testing station in Norway.

However serious losses can occur when SIV simultaneously infects pigs with other swine diseases or when infection occurs in sows during the late stages of pregnancy (Fablet, Marois-Crehan et al. 2012). A study reported that co-infection with *Mycoplasma hyopneumoniae* (Mhp) exacerbated the clinical effects of H1N1 infection (Deblanc, Robert et al. 2013). Wesley (2004) observed stillbirths in naturally infected gilts after challenge with live H3N2 SIV at 80 to 82 days of gestation. The average percentage of stillbirths was 22% per litter while the control gilts (also naturally infected but not challenged with live H3N2 SIV) had no stillbirths. Furthermore, abortions can also occur when sows are infected with new emerging strains of SIV (Gumbert, Froehlich et al. 2020).

2.7.2 Productivity losses

The productivity losses caused by SIV infection include decreased feed conversion efficiency (FCE) and slower growth in pigs. Er, Lium et al. (2014) recorded an outbreak of H1N1pdm09 in a Norwegian boar station and analysed the infection on production performance in the resident pigs. Their study showed that seropositive and virus-positive pigs had overall reduced ($P < 0.05$) growth performance compared to seronegative pigs, even though the feed intake was not decreased. For seropositive pigs, the negative effect on growth performance was seen during growth from 81 to 100 kg (GF3), whereas feed conversion efficiency (FCE) was reduced requiring an extra 0.029 kg of feed for every 1 kg of weight gain and the average daily growth (ADG, weight gain in kg/day) decreased an average of 0.015 kg/day. For virus-positive (with RT-PCR test) pigs, infection reduced the ADG by 0.058 to 0.015

kg/day and also reduced the FCE (an extra 0.058 to 0.125 kg of feed required for each kg of weight gain). Thus, infection resulted in an additional 2.3kg and 5.9-8.0kg feed for seropositive pigs and virologically positive pigs to reach 100kg bodyweight, respectively. The virus-positive pigs also took an extra 1.6 to 2.4 days to reach 100 kg bodyweight. This delay in reaching market weight would also increase the cost of the disease.

Er, Skjerve et al. (2016) also evaluated the marginal effects of infection of H1N1pdm09 in Norwegian pigs. They estimated that a batch of 150 infected pigs would consume an extra 835 (fifth percentile) to 1,350 kg (95th percentile) feed and take 194 (fifth percentile) to 334 (95th percentile) more pig days to reach expected body weights than for an uninfected batch of 150 pigs. They also found that infection in the late stage of fattening could induce the greatest losses since a pig infected during GF3 required more feed and had a protracted production time compared to pigs infected when they were younger.

2.8 Impacts on public health

2.8.1 Swine-source influenza outbreaks and its prevalence in human

Swine influenza viruses have a distinct impact on the potential for pandemic influenza in humans with 19 influenza A reassortants emerging in humans since 1918. Of these, three were predominantly zoonotic swine influenza variants (Bui, Chughtai et al. 2017). Several swine-to-human spillover infections have been reported in China, as well as in other countries. One child infected with swine influenza H3N2 virus was reported in Hong Kong in 1999 (Gregory, Lim et al. 2001). Zu, Dong et al. (2013) reported a human case infected by European avian-like swine H1N1 influenza virus in Jiangsu province, with the same virus being isolated from the patient's backyard pigs.

Killian, Swenson et al. (2013) investigated an outbreak of H1N1 at an Ohio county fair in the USA in 2007 and detected a triple-reassortant swine H1N1 influenza virus that had infected both people and pigs.

Human-adapted SIVs can result in pandemic circulation. The H1N1pdm09 affected 10-20% of humans globally and was commonly seen as the "seasonal flu" in humans (Short, Richard et al. 2015). In Mexico in the period 2007-2008 12.9 and 3.22% of pig farm workers were positive to H3N2 and H1N1 SIV, respectively (Lopez-Robles, Montalvo-Corral et al. 2012). Ma, Anderson et al. (2015) reported that in China 17.3 and 7.0% of workers in piggeries and elsewhere, respectively were also seropositive to the swine H3N2 virus. However, cross-reactions between antibodies against human seasonal H3N2 and swine H3N2 may have introduced bias into these studies/findings. These studies did not rule out this possibility, and in another study, the authors found seropositivity against seasonal H3N2 virus was a significant risk factor for seropositivity to swine H3N2 virus (Ma, Anderson et al. 2015).

2.8.2 Pathogenicity and transmission to humans

Although several human deaths have resulted from SIV infection (Tang, Shetty et al. 2010, Short, Richard et al. 2015), the majority of the SIV human infections are mild and indistinguishable from other seasonal influenza virus infections. The influenza H1N1pdm09 and the H3N2 variants in the USA are the most recent swine-origin influenza viruses. Human mortality of influenza H1N1pdm09 was approximately 29 deaths per 100,000 infections, and among the 350 human cases of the H3N2 variants, only one patient with unspecified concurrent diseases died (Tang, Shetty et al. 2010, Short, Richard et al. 2015).

2.8.3 Infection pathways: risk factors for human infection

The most common pathway for the swine-to-human spread of SIV is exposure to live pigs. A study reported that exposure to pigs increased the chance of humans being infected with H3N2 SIV (OR=3.05, 95%CI: 1.65–5.64) and working in large breeding herds also increased the likelihood of detecting anti-SIV antibodies in pig farm workers (OR=3.98, 95%CI: 1.00–15.86) (Lopez-Robles, Montalvo-Corral et al. 2012). A study in the USA reported that there were spatio-temporal associations between the number of pig farms within counties and the timing of human flu cases, with peak number of cases during years when SIV was present, indicating transmission between pigs and humans (Lantos, Hoffman et al. 2016).

2.8.4 Prevention of spillover of SIVs to humans

As the circulation of influenza A viruses among pigs and humans is very complicated in terms of the interaction of the two species in different ecosystems, it is difficult to recommend effective measures to prevent the transfer of infection from pigs to humans. Dorjee, Revie et al. (2016) used mathematical modelling to demonstrate that minimizing influenza transmissibility at the pig-human interface through good personal hygiene, avoiding direct contacts with sick pigs, and targeted vaccination of swine workers with protective vaccine strains had significant beneficial effects on reducing spillover to humans. They also evaluated different strategies to minimize the duration and size of outbreaks if a spillover event happened, and they suggested that early detection and effective quarantine in humans had the greatest impact on the control of influenza spread. Their findings support putting more emphasis on the early detection of SIVs with pandemic potential in pigs, and hence the need for strengthening the monitoring of gene recombination among SIVs.

2.9 Control measures for influenza in pigs

2.9.1 Vaccination

Vaccination against SI may protect pigs from infection and is commonly used in sows because it is believed piglets are protected through maternal immunity to homologous influenza A strains (Pardo, Wayne et al. 2019). Allerson, Deen et al. (2013) demonstrated that vaccination of sows could significantly reduce SIV transmission among piglets; however, there are several challenges with SIV vaccination. Firstly, as homologous antibody against circulating strains is vital for the efficacy of vaccination in the field, it is critical to vaccinate with the current circulating strains. However, as different strains are commonly found in herds throughout the world, the failure of vaccination to induce protective immunity by not incorporating homologous local infecting strains in the vaccine cannot be ignored. Secondly, MDA may interfere with immunity against infection with homologous SIV strains in piglets. A study reported that MDA in piglets could result in a prolonged shedding period of the virus when the piglets were subsequently infected with homologous SIV strains (Rose, Herve et al. 2013).

2.9.2 Surveillance for swine influenza viruses

Surveillance programs for SIV have been developed and implemented in many countries. In the USA, the aims of SIV surveillance include the protection of public health. However, detection, discovery, and sharing of virus isolates to facilitate updates for vaccines, refine diagnostic assays, and determine the distribution of new influenza strains in pigs to inform further policy decisions are also advantages of this surveillance (Corzo, Culhane et al. 2013, Kaplan, DeBeauchamp et al. 2015). In Europe, the European Surveillance Network for Influenza in Pigs (ESNIP) was established in 2001. This was designed to "increase the knowledge of the epidemiology and evolution of swine influenza virus in European pigs". Most of the funds associated with this network have been directed towards undertaking research on the antigenic and genetic characterization of field isolates of SIV (Detmer, Gramer et al. 2013).

For the purpose of preventing potential pandemic human influenza, it is valuable to monitor genetic drift, co-infection with different SIV subtypes on pig farms and emerging new reassortants of SIVs (Simon, Larsen et al. 2014, Rajao, Walia et al. 2017). Thus, subtyping and gene sequencing of field strains and isolation of live strains is required.

Virological tests are often not sensitive in the field because excretion of SIV is transient in infected pigs (Van Reeth, Gregory et al. 2003, Hemmink, Morgan et al. 2016) resulting in many affected pigs returning a virus-negative outcome. Furthermore it is often difficult to culture SIVs and therefore subtype them when the viral load in samples is low. For example, Lopez-Robles, Montalvo-Corral et al. (2014) reported that even when clinical signs were present in 22 of 25 pigs that were positive to the matrix gene of influenza A, only isolates from 6 affected pigs were able to be subtyped by RT-PCR.

It is recommended that risk-based surveillance strategies are implemented to improve the efficiency of SIV surveillance. Risk-based surveillance is designed to detect pathogens or infections in the most likely places, herds or individuals, and thus can improve the sensitivity of the surveillance system leading to more efficient use of resources and time (East, Wicks et al. 2013). Risk-based surveillance relies on knowledge about the diseases' epidemiological characteristics, including the determinants for its spread and transmission (Stark, Regula et al. 2006, Oidtmann, Peeler et al. 2013).

Surveillance for influenza A viruses, including surveillance for SI, is in place in many countries (Kaden, Lange et al. 2008, Simon, Larsen et al. 2014, Vincent, Awada et al. 2014, Kaplan, DeBeauchamp et al. 2015). However, there is still room for improvement of SIV surveillance. Firstly, SI surveillance in key areas is insufficient. The surveillance capacity varies between countries, with many undeveloped countries having limited resources hindering their surveillance capacity. Secondly, the existing surveillance programs have not generated sufficient knowledge on the epidemiological features of SIV in different ecosystems. Thirdly, although passive surveillance is common in many countries, well-designed active surveillance is still rare. Passive surveillance may introduce bias in evaluating the presence and distribution of SIVs. Lastly, while more reassortants have been confirmed and compared with phylogenetic analysis, the relevant risk factors for infection remain unclear (Trevennec, Cowling et al. 2011, Vincent, Awada et al. 2014, Nelson, Viboud et al. 2015).

2.10 Social network analysis (SNA) and its role in SI

Social network analysis has been used to investigate animal movements allowing implementation of more effective disease control in livestock populations (Dubé, Ribble et al. 2011). Social network analysis involves a multidisciplinary approach that focuses on investigating the relationships between interacting units (Wasserman and Faust 1994). The idea of the social network was firstly explored by psychologists in their studies on the flow of information through groups. Similar to the spread of news between people, contagious pathogens can be transmitted between animals, premises and places via the contacts between them (Earley, Buckham Sporer et al. 2017, Lee, Polson et al. 2017, Rossi, De Leo et al. 2017).

Due to the mathematical nature of SNA, some of the terms are abstract notions. The definitions of some terms used in SNA in epidemiology are introduced in Table 2.1.

Table 2.1 Definitions of social network analysis terms used in epidemiology*

Parameter	**Definition**
Node	A node refers to a unit of interest in a network (Dube, Ribble et al. 2009).
Edge	An edge represents a contact between individuals in the susceptible population (Shirley and Rushton 2005).
Small-world	When the nodes in a network are highly clustered, and the nodes are connected with each other with short paths in general, the network can be defined as a small-world network (Watts and Strogatz 1998).
One-mode network	The nodes in the network are considered as belonging to the same category (Wasserman and Faust 1994). For example, a group of farms having animal movement between them.
Two-mode network	The nodes in the network are considered as belonging to two different sets, and the structure of the network can be measured on these two sets (Wasserman and Faust 1994). For example, a group of farms having animal movement between them, but the farms are owned by different companies.

Parameter	**Definition**
Edge density	A value reflecting the density of the network that can be calculated using the equation: L/k(k - 1), where L is the number of exiting edges and k is the number of nodes in the network (Wasserman and Faust 1994).
Diameter	The longest geodesic between any pair of nodes in the network (Wasserman and Faust 1994).
Average path length	For any two given nodes, the shortest path between them over the paths between all pairs of nodes in the network (Dube, Ribble et al. 2009)
Degree	The total number of contacts of a county to other counties in the network. A higher degree means more connection to other nodes in the network (Marquetoux, Stevenson et al. 2016).
Betweenness	The frequency by which a node falls between pairs of other nodes on the shortest path connecting them (Dube, Ribble et al. 2009). Betweenness is a measure of centrality used to quantify a node’s potential to ‘control’ the flow or curtail paths within a network (Marquetoux, Stevenson et al. 2016).

Parameter	**Definition**
Closeness	The sum of the shortest distances (not geographical, but path length) from a source livestock operation to all other reachable operations in the network (Shirley and Rushton 2005).
Clustering coefficient	This measure assesses the degree to which nodes tend to cluster together. It represents the proportion of one node's neighbours, who are also neighbours to another (Watts and Strogatz 1998).
Giant weakly connected component (GWCC)	The weakly connected component is the undirected subgraph in which all nodes are linked, not taking into account the direction of the links (Robinson and Christley 2007). GWCC is the largest weak component in the network (Dube, Ribble et al. 2009).

* Part of this table has been published in the manuscript outlined in full in Chapter Four of this thesis: Li, Y., Huang, B., Shen, C., Cai, C., Wang, Y., Edwards, J., Zhang, G., & Robertson, I. D. (2020). Pig trade networks through live pig markets in Guangdong Province, China. Transboundary and emerging diseases, 67(3), 1315–1329. https://doi.org/10.1111/tbed.13472.

Animal movement plays a significant role in the spread of most infectious diseases (Martin, Zhou et al. 2011, Guinat, Relun et al. 2016). A study on bovine tuberculosis (bTB) in East Africa demonstrated that the network of cattle movement had a significant impact on the TB infection status of cattle herds. The analysis of associations indicated that the herd's degree, betweenness, and closeness were positively correlated to infection. In contrast, the fragmentation index was negatively related to a herd’s bTB infection status (Sintayehu, Prins et al. 2017). Focusing on animal movement and farm-level parameters derived from animal movement, Scharrer, Widgren et al. (2015) developed a framework to select farms for risk-based surveillance for contagious diseases such as bovine viral diarrhoea (BVD) in cattle farms, and they validated its use with data collected from a BVD surveillance programme in Switzerland. Another study on cattle movements in the Uruguayan cattle industry found an extreme high level of heterogeneity in movement patterns. The study suggested disease control and surveillance should target specific farms to contain disease outbreaks (VanderWaal, Picasso et al. 2016).

Social network analysis was used in veterinary epidemiological studies for the first time in 2002 (Webb and Sauter-Louis 2002). Since then, it has been used in several aspects of animal disease control. One of the most significant purposes of SNA is to identify the key players in an animal movement network. A study on pig and pork movement in border areas between Kenya and Uganda (a trading network) identified several key nodes for ASF spread between the two countries (Lichoti, Davies et al. 2017). A study of the cattle movement network in Denmark demonstrated a large degree of heterogeneity and the authors suggested that the livestock markets had a higher risk of receiving pathogens than did farms and recommended that network analysis should play an important role in disease control programs (Bigras-Poulin, Thompson et al. 2006).

Social network analysis has also been applied in modelling the potential for transmission of a pathogen within a network (Marquetoux, Heuer et al. 2016). Modelling can be used to predict how many premises would become infected if an epidemic was to spread through the studied network (Dube, Ribble et al. 2011), as well as to evaluate the effects of different interventions on the control of the epidemic and hence SNA can be used to identify the most effective methods to apply to control the disease of interest (Gates and Woolhouse 2015, Marquetoux, Stevenson et al. 2016).

Animal movements, including the introduction of live animals for breeding or fattening, transporting livestock to slaughterhouses, selling animals in livestock markets and attending livestock shows, are the most commonly used data for SNA (Kiss, Green et al. 2006, Martin, Zhou et al. 2011, Marquetoux, Stevenson et al. 2016, Lee, Polson et al. 2017). Besides the movement of live animals, contaminated personnel, vehicles and feed can also be involved in the spread and introduction of new diseases into premises (Rossi, De Leo et al. 2017). Some SNA studies (Brennan, Kemp et al. 2008, Rossi, De Leo et al. 2017) have explored the indirect contacts between premises, including sharing equipment and visiting personnel, and have highlighted that SNA may help in the development of disease control and prevention measures.

The movement network of live pigs in China is complicated. The provinces in the centre, southwest and northeast of China, such as Henan, Sichuan and Liaoning, have large areas of intensive cropping and massive pig populations (Ministry of Agriculture and Rural Affairs 2018), whilst the majority of the big cities are located in the provinces in the east and south of China. This separation of the pig and human populations results in the need for long-distance transport of live pigs between provinces. Furthermore as Chinese people have a preference for meat from freshly slaughtered animals, as opposed to chilled or frozen meat (Lin, Zhang et al. 2017), live pigs are usually slaughtered in slaughterhouses located in or near the large cities. In south China there are live pig markets in cities, such as Guangzhou and Foshan. Pigs sourced from distant farms are traded in these markets. The movement network of live pigs via these markets and the trade practices in these markets are largely unknown, even though it is well known that live animal markets can play a key role in the rapid spread of epidemics in animals in China (He, Liu et al. 2014, Zhou, Li et al. 2015, Cao, Jin et al. 2018). To better understand the risk of spreading SI and other diseases between areas via these live pig markets, SNA on the movement of pigs in the local market trading system is needed and this formed one aspect of the research reported in this thesis (Chapter Five).

Establishing an effective control strategy for SI in China requires a detailed understanding of the epidemiology of the disease. However, very little was known about the prevalence and risk factors of SI, and the biosecurity gaps in local pig farms before this study. Given the zoonotic risk of SI, it is vital to understand the interactions between pigs and pig industry workers. Previous studies have indicated that the movement of livestock and environmental and meteorological factors impact upon the spread of animal diseases (Gilbert, Golding et al. 2014, Sintayehu, Prins et al. 2017). However, no previous studies had explored the roles of these factors on SI in south China, where influenza is prevalent in the massive populations of pigs, poultry and humans. In the next chapter the findings of a study to determine: the husbandry and biosecurity practices adopted, and the farm-level prevalence, spatial distribution and farm-level risk factors for SI infection in pig farms in Guangdong Province are reported.

CHAPTER 3：　Prevalence, distribution and risk factors of farmer reported swine influenza infection in Guangdong Province, China

Preface

Swine influenza is endemic in the Chinese pig population; however most studies conducted in China on SI have previously only focused on monitoring the changes in the gene sequences of SIV isolates. Prior to the research reported in this thesis there was little information available on the prevalence of the disease in China, and factors linked to its spread between Chinese pig farms. This lack of epidemiological knowledge about SI in China has been a critical obstacle to the control of SI in the country. In this chapter, the husbandry, management and biosecurity practices adopted by pig farmers in Guangdong Province are described. Guangdong Province in south China was selected for this research as it has been considered as a hot spot for influenza containing large areas of wetlands, live animal markets and large human and pig populations, and previous studies have shown that there are many SIV reassortants circulating in the pig population of this province (Yang, Chen et al. 2016). The prevalence of farmer perceived SI infection at the farm-level and the associated risk factors were also explored to address the identified deficits in the understanding of SI in Guangdong Province, China.

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Overall percentage (%)	60
Signature	Date: 15/06/2020

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By signing the Statement of Contribution, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis.

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Overall percentage (%)	5
Signature	Date:15/06/2020

Abstract

A cross-sectional study was undertaken to better understand the husbandry, management and biosecurity practices of pig farms in Guangdong Province (GD), China to identify risk factors for farmer reported swine influenza (SI) on their farms. Questionnaires were administered to 153 owners/managers of piggeries (average of 7 from each of the 21 prefectures in GD). Univariable and multivariable logistic regression analyses were used to identify risk factors for farmer' reported SI in piggeries during the six months preceding the questionnaire administration. The ability of wild birds to enter piggeries (OR 2.50, 95% CI: 1.01-6.16), the presence of poultry on a pig-farm (OR 3.24, 95% CI: 1.52-6.94) and no biosecurity measures applied to workers before entry to the piggery (OR 2.65, 95% CI: 1.04-6.78) were found to increase the likelihood of SI being reported by farmers in a multivariable logistic regression model. The findings of this study highlight the importance of understanding the local pig industry and the practices adopted when developing control measures to reduce the risk of SI to pig farms.

3.1 Introduction

Swine influenza (SI) is a respiratory disease of pigs caused by swine influenza virus (SIV) – a type A influenza virus (Brown 2000). Typical clinical signs include coughing, labored breathing, nasal discharge, sneezing and pyrexia (Kothalawala, Toussaint et al. 2006). Since SI is a highly contagious disease, the morbidity on infected farms is often nearly 100%, although mortality is usually very low. The infection is often mild, resulting in low direct losses from the disease (Er, Lium et al. 2014), although serious losses can happen when SIV simultaneously infects pigs with other pathogens or when infection occurs in sows during late pregnancy (Fablet, Marois-Crehan et al. 2012). Wesley (2004) reported 22% stillbirths in naturally infected gilts after infection with H3N2 SIV at 80 to 82 days of gestation. Abortions can also occur when sows are infected with new strains of SIV (Choi, Goyal et al. 2002). Swine influenza is also a potential threat to human health (Ito, Couceiro et al. 1998).

Swine influenza is one of the most ubiquitous diseases circulating in the global pig population. Corzo, Culhane et al. (2013) reported a 90.6% herd prevalence in USA using a real-time reverse transcription polymerase chain test and a cross-sectional study in northern Mexico reported that more than 50% of pigs from commercial farms were seropositive to H1 or H3 subtype SIV (Lopez-Robles, Montalvo-Corral et al. 2014). Swine influenza is also widespread in Europe. An analysis of historical surveillance data in Norway showed that the national herd seroprevalence of influenza

A(H1N1)pdm09 virus was around 43%, and the individual pig seroprevalence of pandemic H1N1 on infected farms was more than 60% (Er, Skjerve et al. 2016). Another study in 2009 reported almost 100% herd-seroprevalence against SIV in 98 randomly selected piggeries in Spain, with 62.3% of individual animals seropositive (Simon-Grife, Martin-Valls et al. 2011). In England, a 52% herd prevalence was reported by Mastin, Alarcon et al. (2011) with the highest individual seroprevalence being 33% in sows.

Swine influenza is endemic in the Chinese pig population, with many subtypes contemporaneously circulating in pig farms. Serological evidence of H1, H3, H4, H5, and H9 influenza viruses has been found in the Chinese pig population (Ninomiya, Takada et al. 2002, Yu, Zhou et al. 2011). Liu, Wei et al. (2011) reviewed the data from 10 years of publications and concluded that the average individual pig seroprevalence to subtypes H1, H3, H5, H7 and H9 were 31.1, 28.6, 1.3, 0 and 2.4%, respectively. Song, Xiao et al. (2010) reported an individual pig seroprevalence of more than 50% for H1 and H3 in commercial farms in Fujian Province. However, no antibody against H5N1 was detected in pigs in Fujian, and while H9 infection was detected it was only at a very low seroprevalence (1% in 2004 and 2.6% in 2007). In Tibet, 52 and 16.9% of pigs were seropositive to H1N1 and H3N2, respectively (Liu, Zhou et al. 2014). Infection with more than one subtype of SIV often occurs in the Chinese pig population. For example, 8.8 and 24% of the pigs tested in Fujian

Province and Tibet, respectively were seropositive for H1 and H3 (Song, Xiao et al. 2010, Liu, Zhou et al. 2014).

China, particularly south China, is considered by some as "the epicenter of influenza" (Liu, Ji et al. 2011), because of the unique ecosystem containing vast wetlands, live animal markets, and one of the largest human and pig populations in the world. Other studies have shown that SI is not evenly distributed in China and is more prevalent in south China (Yu, Zhang et al. 2009). Unfortunately, husbandry, management and biosecurity practices adopted on pig farms in China are rarely described and no information is available on potential risk factors for SI infection in pig farms in China. The objectives of this study were to describe: the herd level prevalence in pig farms reporting SI infection; the distribution of infection; the husbandry, management and biosecurity practices adopted on the surveyed pig farms; and the putative risk factors for SI in Guangdong Province.

3.2 Materials and methods

3.2.1 Sample strategy

he study was conducted in Guangdong Province in July and August 2015. The sampling frame was the client lists of 10 private consultants who were offering veterinary services to pig farms in all 21 prefectures within the province. The average number of clients (piggeries) per prefecture for the consultants was 80. The veterinary consultants used a random number process to randomly select piggeries from their complete client lists for sampling. On average 7 farms were randomly selected from each of the 21 prefectures in the province (total of 153 pig farms surveyed) (Fig. 3.1). Four of the consultants provided details on the number of farms serviced and the number of pigs on these farms in 15 prefectures.

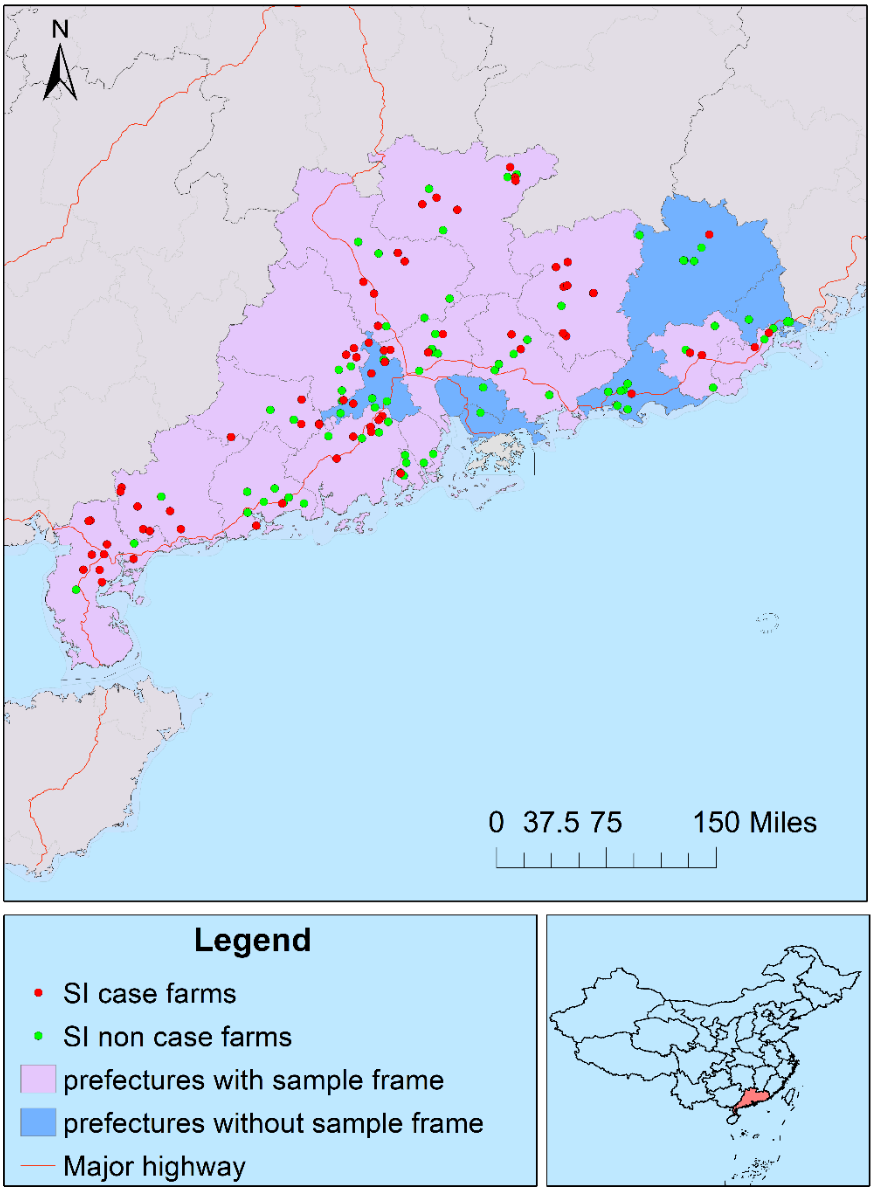

Figure 3.1 Sampled pig farms in Guangdong Province

3.2.2 Data collection

A questionnaire was designed and administered to collect information about husbandry, management, trade and biosecurity practices, and interfaces between pigs and other animal species, including humans. The farmers were asked if a swine flu-like syndrome, such as coughing, nasal discharges or sneezing, had been seen in their pigs in the six months prior to the questionnaire being administered. Data were collected on when this event occurred, its duration, mortality levels, and whether it was confirmed by diagnostic tests and/or by a veterinarian. The questionnaire was pretested on 12 farms and subsequently revised. The final questionnaire contained 84 questions and the average response time to complete was 30 minutes. The questionnaires were administered to piggery owners/managers by the consultants in a face-to-face setting. The consultants were trained in delivering the questionnaire by the authors before administering the survey. The questionnaire and its delivery had been approved by the South China Agriculture University Human Ethics Committee.

3.2.3 Data analyses

Using information collected from the piggery and from the consultants, a case was defined as a farm that had contained pigs with SI-like clinical signs in the six-months preceding the questionnaire administration and which also met at least one of the following criteria:

- The outbreak lasted less than 30 days on the farm;
- The morbidity was higher than 10%;

- The case fatality rate was less than 5%;
- The outbreak was diagnosed as SI infection by a veterinarian or from laboratory samples.

70 farms that met the criteria were defined as case farms. Among these, 19 had the epidemic diagnosed by a professional (12 by a on–farm veterinarian and 7 by a diagnostic laboratory). The remaining 51 case farms all had SI-like clinical signs in pigs and met at least 1 of the first 3 criteria (19 farms met 1 of the criteria; 29 met 2 of the criteria; and 3 case farms met all of the first 3 criteria).

The herd prevalence was estimated only in the 15 prefectures with a known sampling frame by weighting in each stratum (prefecture) in Microsoft Excel (Redmond, WA, USA) using the method of Dohoo, Martin et al. (page 35-37, 2010). Maps were developed with ArcGIS 9.3 (ESRI Inc., Redlands, CA, USA) to show the location of the affected and non-affected piggeries. Statistical descriptions of the husbandry, management, trading and biosecurity practices were conducted with Microsoft Excel (Redmond, WA, USA) and R software (version 3.0.2). The total number of cases per month was calculated and the number of cases in each month was illustrated by constructing a histogram using Microsoft Excel.

Data collected from the 153 pig farms were used to identify putative risk factors for SIV infection in the 6 months preceding the administration of the questionnaire. Univariable and multivariable logistic regression analyses were done using SPSS (SPSS Inc., IBM Corporation, Somers, NY) version 19 to identify risk factors for farmer' reported SI infection in their piggery. Ten risk factors were excluded from the multivariable logistic regression analysis due to collinearity and two risk factors were excluded due to similarity to other risk factors. Factors (12) with P-values < 0.2 in the univariable logistic regression analyses were offered to a multivariable model. A stepwise backward method was used to generate a final model with variables retained when the P-value of the likelihood ratio test was < 0.05. Interactions between factors in the final model were examined for statistical significance. The goodness of fit of the final model was tested using the Hosmer-Lemeshow test. Area under the curve (AUC) was also calculated with SPSS.

3.3 Results

3.3.1 Herd prevalence

Of 153 surveyed farms, 70 (46%) were defined as cases. Using the data from the 15 prefectures where the total number of farms in the sampling list was known, the herd prevalence of farmer' reported SI infection in the preceding 6 months was 58% (95% CI: 48 - 68%), after adjusting for the sample weights in each stratum.

Temporal distribution of SI infection

Fifty-nine of the 70 case farms reported the onset dates or months of the SI-like infection. For the data collected (January to August, 2015) the most cases of SI-like infection were observed from March to May (Fig. 3.2).

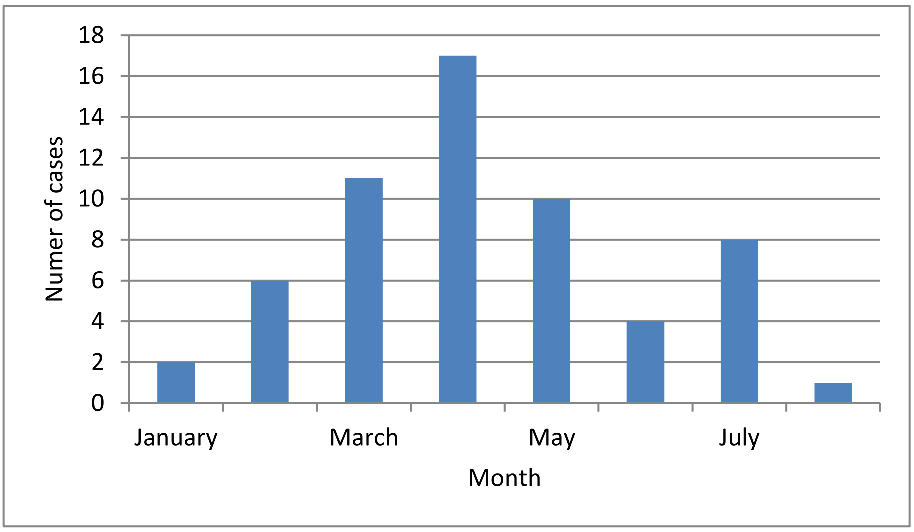

Figure 3.2 Temporal distribution of farmers-perceived SI cases between January and August 2015 in Guangdong province

3.3.2 Demographic, management and husbandry practices of pig farms

The demographic profile of farms participating in the study and the on-farm husbandry and management practices are summarized in Table 3.1.

The majority (86%) of the farms involved in this study were farrow to finish pig farms (breed, grow and fatten pigs and then send them to a slaughterhouse), 11% were farrow to wean farms (sell gilts or weaners to other farms for breeding or fattening purposes), and 3% of the surveyed farms were fattening farms (purchased weaners to fatten). Approximately half (46%) were categorized as small farms (< 2000 head). Farrow to wean farms had larger populations than farrow to finish and fattening farms and were more likely to record production information and employ a veterinarian as a full-time worker on the farm.

Table 3.1 Demographic profile and husbandry practices of the 153 pig farms participating in the study categorized by herd size and farm type

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
N (%)		131 (86%)	18 (11%)	4 (3%)	71 (46%)	82 (54%)	
Total pig population (mean ± SD)		2810 ± 2690	12511 ± 17029	1260 ± 424	1098 ± 970	6347 ± 17673	
Duration of operation (Years)	=< 10	76%	83%	100%	76%	78%	77%
	> 10	24%	17%		24%	22%	23%
Keep production records	Yes	76%	100%	75%	72%	85%	79%
	No	24%		25%	28%	15%	21%
Total employees (mean ± SD)		12.0 ± 15.0	54.9 ± 63.8	3.8 ± 1.0	5.3 ± 5	26.8 ± 73.8	
Full-time veterinarian employed on farm	Yes	47%	94%	25%	34%	68%	52%
	No	53%	6%	75%	66%	32%	48%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Employees live on the farm	Yes	93%	94%	100%	90%	96%	93%
	No	2%	6%		3%	1%	2%
	Not always	5%			7%	2%	5%
Accommodation area for staff adjacent (< 10 meters) to buildings housing pigs	Yes	31%	11%	25%	39%	20%	29%
	No	69%	89%	75%	61%	80%	71%

3.3.3 Practices for introduction and selling of pigs

The practices for the introduction and selling of pigs on surveyed farms are presented in Table 3.2. Fattening farms introduced more pigs and at more frequent intervals (6.5 times per year with about 1200 head in total) than farrow to finish farms (1.8 times per year with about 140 head) and breeding farms (1.5 times per year with about 70 head); farrow to wean farms sold more pigs more frequently (a total of 27600 head sold 210 times per year) than farrow to finish farms (5327 pigs sold 46 times per year) and fattening farms (800 pigs sold 6 times per year). Of the interviewed owners/managers, 89% would contact farrow to wean farms directly when they needed new stock, but 5% of them would use agents ("middle-men") and 1% of them would attend a live pig market for replacement stock. When selling pigs, less than half of the farms (42%) would sell all the pigs in a pen at one time. On 30% of the visited farms, buyers would participate in selecting pigs for purchase and their subsequent loading onto trucks.

Table 3.2 The introduction of live pigs and selling practices of farms participating in the study

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Introduced pigs in the year preceding the survey	Yes	61%	28%	50%	66%	50%	57%
	No	39%	72%	50%	34%	50%	43%
Source of introduced pigs	Breeding farms	88%	100%	100%	90%	89%	89%
	Middle men	6%			6%	5%	5%
	Live pig market	1%				2%	1%
	Others	5%			4%	5%	4%
New pigs are quarantined when introduced	Yes all the time	62%	100%	50%	45%	85%	65%
	Sometimes	14%	0%	0%	18%	6%	12%
	Never	24%	0%	50%	37%	8%	23%
Measures undertaking during quarantine in farms which adopted quarantine practices	Observe for signs of illness only	52%	14%	50%	61%	38%	50%
	Observe pigs and do diagnostic tests	37%	86%	0%	24%	55%	40%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
	Observe pigs and occasionally collect samples for testing	11%	0%	50%	15%	6%	11%
Sell all the finishing pigs in an individual pen	Yes all the time	43%	33%	50%	32%	51%	42%
	Sometimes	30%	33%	50%	28%	33%	31%
	Never	27%	33%		39%	16%	27%
Who selects and loads pigs for sale	Workers from the farm only	68%	94%	50%	66%	74%	70%
	Buyers only	11%			15%	5%	10%
	Both	21%	6%	50%	18%	21%	20%
People loading pigs change their clothes before entering the piggery to select and load pigs	Yes	46%	6%	75%	61%	26%	42%
	No	54%	94%	25%	39%	74%	58%
People loading pigs change their boots before entering the piggeries to select and load pigs	Yes	30%	6%	50%	37%	21%	28%
	No	70%	94%	50%	63%	79%	72%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Ever seen half loaded truck (presence of other farm pigs on truck) before loading	Yes	13%			18%	5%	11%
	No	77%	100%	75%	65%	93%	80%
	Not sure	10%		25%	17%	2%	9%
Number of times pigs were introduced in the year preceding the survey (mean ± SD)		1.8 ± 1.2	1.8 ± 0.8	6.5 ± 7.8	1.8 ± 3.4	1.9 ± 2.8	
Number of pigs introduced in the year preceding the survey (mean ± SD)		138 ± 366	70 ± 19	1211 ± 1682	104 ± 712	216 ± 974	
Number of times pigs were sold during the year preceding the survey (mean ± SD)		45 ± 70	201 ± 301	5.5 ± 6.4	21 ± 63	96 ± 328	
Number of pigs sold during the year preceding the survey (mean ± SD)		5228 ± 10413	25999 ± 32120	800 ± 1131	1959 ± 5069	12109 ± 40335	

3.3.4 Biosecurity management practices on farms

The biosecurity practices of farms participating in the study are presented in Table 3.3. In general, breeders adopted better biosecurity management practices than fattening and farrow to finish farms, and similarly, larger farms had better biosecurity than smaller ones. However, on average, only about 70% farms had a vehicle disinfection drive-through tyre wash at the front-gate, only about half of the surveyed farms required all vehicles from outside to be disinfected. Dogs, cats and poultry were commonly present (more than 50%) on pig farms. In 46% of the farms with dogs/cats, the dogs/cats could contact pigs directly. Of the 86 farms which also kept some poultry, 69% of them purchased poultry from live bird markets and 67% of farms had the same worker feed both the pigs and the poultry. Approximately 90% of the farms (141) had a pond on their farms, with 18% of them using the pond water for flushing waste from their piggeries and two used pond-water as pig drinking water. Swill was fed to pigs in only 3.9% (6) of the surveyed farms.

Table 3.3 Biosecurity practices adopted in the participating farms

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Disinfection pool for trucks at the farm entrance	Yes	69%	94%	75%	59%	83%	72%
	No	31%	6%	25%	41%	17%	28%
Disinfection of vehicles from outside	Yes, always	50%	89%	50%	37%	71%	55%
	No or sometimes	50%	11%	50%	63%	29%	45%
Not allow visitors to enter the piggery	Yes	73%	89%	25%	69%	77%	73%
	No	27%	11%	75%	31%	23%	27%
Dogs/cats present on farm	Yes	77%	50%	100%	82%	68%	75%
	No	23%	50%		18%	32%	25%
Dogs/cats can have direct contact with pigs [a]	Yes	46%	44%	50%	49%	43%	46%
	No	54%	56%	50%	51%	57%	54%
Dogs/cats can have direct contact with pig	Yes	38%	38%	25%	38%	36%	37%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
feed or drinking water [a]	No	62%	62%	75%	62%	64%	63%
Feed raw poultry meat or pork to dogs/cats [a]	Yes	29%	12%	50%	38%	18%	28%
	No	71%	88%	50%	62%	82%	72%
Poultry present on farm	Yes	59%	33%	100%	75%	41%	57%
	No	41%	67%		25%	59%	43%
The same person(s) feeds both pigs and poultry [b]	Yes	68%	67%	50%	69%	64%	67%
	No	32%	33%	50%	31%	36%	33%
Source of poultry [b]	Live bird markets	71%	67%	25%	75%	59%	69%
	Nearby villages	11%		25%	8%	15%	10%
	Breeder poultry farms	10%	17%	25%	10%	15%	12%
	Breed themselves	8%	17%	25%	8%	12%	9%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Pond present on farm	Yes	88%	100%	75%	81%	97%	89%
	No	12%		25%	19%	3%	11%
Pond water used as a source of drinking water for pigs [c]	Yes	1%	6%			3%	1%
	No	99%	94%	100%	100%	98%	99%
Pond water used to flush piggeries [c]	Yes	17%	22%		20%	16%	18%
	No	83%	78%	100%	80%	84%	82%
Netting used to prevent access of birds to piggery	Yes	10%	24%	25%	7%	17%	12%
	No	90%	76%	75%	93%	83%	88%
Wild birds able to enter piggery	Yes	45%	47%	50%	55%	36%	45%
	No	23%	41%		16%	32%	25%
	Not sure	32%	12%	50%	28%	31%	30%

Explanatory variable	Level	farm type			farm size (head)		Total
		farrow to finish	farrow to wean	fattening	< 2000	≥ 2000	
Is swill fed to pigs?	Yes	4%		25%	6%	2%	4%
	No	96%	100%	75%	94%	98%	96%

[a] Only conducted with the farms having dogs/cats present on the farm.

[b] Only conducted with farms keeping poultry on farm.

c Only conducted with the farms having a pond on the farm.

3.3.5 Risk factor analysis

Among the 84 questions, 52 factors were analyzed and 24 factors were significantly associated ($p < 0.20$) with farmer' reported SI infection in the univariable logistic regression analyses (Table 3.4). The results of the multivariable logistic regression analysis are presented in Table 3.5. In the final multivariable logistic regression model, piggeries that did not prevent the entry of wild birds, raised poultry or did not have a disinfection pool at the piggery entrance were more likely to have an outbreak of SI in the 6 month period preceding the administration of the questionnaire (OR = 2.50, 95%CI: 1.01-6.16; OR = 3.24, 95%CI: 1.52-6.94; OR = 2.65, 95%CI: 1.04-6.78; respectively) (Table 3.5). The Hosmer–Lemeshow test of goodness of fit ($p = 0.73$) and the AUC (0.73; 95%CI: 0.65-0.81) indicated that the model fitted the data well and had a medium predictive ability.

Table 3.4 Results of the analysis by univariable logistic regression for owner reported Swine Influenza infection in piggeries

ID	Risk factors	P-value	OR (95%CI)
1	Less than 10 years of operation	0.056	2.18 (0.98, 4.85)
2	Less than 2000 head inventory	0.006	2.5 (1.3, 4.8)
3	No quarantine implemented	0.084	1.78 (0.93, 3.43)
4	Don’t sell all finishing pigs in one pen every time	0.121	1.68 (0.88, 3.21)
5	People loading pigs do not change clothes before entering piggery	0.001	3.28 (1.68, 6.41)
6	People loading pigs do not change boots before entering piggery	0.056	2.26 (0.98, 4.1)

ID	Risk factors	P-value	OR (95%CI)
7	Workers loading pigs do not conduct spray disinfection to their clothes/boots after loading trucks	0.006	2.72 (1.34, 5.5)
8	No production records kept	0.013	2.81 (1.24, 6.34)
9	No veterinarians among employees	0.002	2.81 (1.46, 5.43)
10	Workers occasionally work in different piggeries	0.001	3.08 (1.58, 6.01)
11	No disinfection of workers before entering the piggery	0.001	4.29 (1.83, 10.04)
12	Without scheduled disinfection of pig pens	0.081	1.86 (0.93, 3.74)
13	Process feed in the piggery	0.115	2.15 (0.83, 5.57)
14	Not separate living area of employees from piggery area	0.083	1.87 (0.92, 3.8)
15	Visitors are allowed to enter the piggery	0.009	2.68 (1.28, 5.62)
16	Dogs/cats on the farm	0.032	2.33 (1.08, 5.05)
17	Poultry on the farm	<0.001	3.96 (1.99, 7.9)
18	Wild birds able to gain entry to the piggery	0.002	4.22 (1.71, 10.4)
19	Wild birds have potential contact with drinking water of pigs	0.006	4.18 (1.5, 11.65)
20	Eat poultry meat on farm	0.017	6.41 (1.39, 29.46)
21	Purchase live poultry to cook on farm	0.024	2.25 (1.11, 4.53)
22	Not using mouth mask/gloves when treating sick pigs	0.005	2.69 (1.35, 5.34)
23	No college graduates employed on the farm	<0.001	5.19 (2.28, 11.83)
24	Introduced pigs in the year preceding the questionnaire	0.196	1.54 (0.8, 2.94)

Table 3.5 Results of the analysis by multivariable logistic regression for owner reported Swine Influenza infection in piggeries

	B	Sig.	OR	95% CI for OR	
				Lower	Upper
Wild birds able to enter piggery	0.92	0.047	2.50	1.01	6.16
Poultry present on the farm	1.18	0.002	3.24	1.52	6.94
The workers are not required to undertake any biosecurity measures, such as changing clothes/boots, having a shower or disinfecting their boots, before they enter the piggery	0.97	0.042	2.65	1.04	6.78
Constant	-1.71				

3.4 Discussion

A high seropositivity of SI at the individual animal level has been reported in previous studies in the Chinese pig population (Song, Xiao et al. 2010, Liu, Wei et al. 2011, Strelioff, Vijaykrishna et al. 2013). However, the herd prevalence of SIV infection in China has rarely been reported and it is likely that the individual animal prevalence is biased through the sampling methodology used. This study found a high farmer-reported herd prevalence (almost 60%) in pig farms in Guangdong Province from January to August 2015. To our knowledge, this is the first study describing husbandry, management and biosecurity practices adopted in Chinese pig farms and identifying risk factors for SI infection in pig farms in China.

This study had several strengths and limitations. Due to the unwillingness of many farmers to allow collection of serum samples from their pigs, we used farmer reported SI infection when analyzing potential risk factors. The clinical signs of SI infection in pigs are similar to other respiratory diseases, including porcine reproductive and respiratory syndrome and infection with Mhp, however the low mortality, short duration and recovery without therapy help in differentiating SI from other respiratory diseases (Detmer, Gramer et al. 2013, Kong, Ye et al. 2014). In this study, the dependent variable, (SI), relied partly upon the farm owners/managers’ knowledge of the disease and partly on epidemiological features or diagnosis of the disease. It is believed that the farmers surveyed should be familiar with SI as it is a commonly seen disease in local pig farms and 93% of farmers visited claimed they knew about SI. Nearly half of the surveyed farms claimed that they had participated in training on swine diseases offered by local official veterinary stations in the preceding year (data not shown). The temporal distribution of farmer reported SI outbreaks highlighted a peak of infection during March to May, which fits well with the SI surveillance results with serum tested by the provincial university laboratory (personal communication with the head of the laboratory). Although a case was identified using a variety of disease effects, it is worth investigating the association between farmer-reported SI infection and the results of laboratory diagnostic tests in future studies. Although the accuracy of farmers’ perception on SI epidemic in a herd hasn’t been evaluated in China, several studies conducted internationally have indicated that pig farmers have a good knowledge on SI (Hernandez-Jover, Taylor et al. 2012, Rabinowitz, Fowler et al. 2013). Data on farmer’ reported SI outbreaks also relies heavily on the willingness of farmers to cooperate in the study, consequently we used the services of veterinary consultants who offer technical support to the local farmers, to increase the response rate. All surveys were administered by the consultants, and none of the randomly selected farmers/managers refused to be involved in the study. Recall bias could be another obstacle for a syndrome survey (O'Neill, Church et al. 2014); however in this study 80% of the interviewed farms had detailed production records documenting the specific onset date (44/70) or month (59/70) of the SI outbreaks experienced.

The univariable logistic regression analysis indicates that there were many (16) significant variables that may be reflective of poor biosecurity. These and the three significant variables from the multivariable logistic regression analysis demonstrate implementation of poor biosecurity practices on many local pig farms. The husbandry and biosecurity practices adopted by local farms indicate several potential pathways for the introduction of SI into the surveyed farms. For example, live pig movement between pig farms is considered a high-risk practice (Brown 2000, Almeida, Storino et al. 2017) with 57% of the visited farms having introduced live pigs in the year preceding the survey. Approximately one-third (35%) of the farms that introduced pigs in the preceding year did not always quarantine these introduced stock, and of those who did adopt some form of quarantine, half of them only used visual inspection for signs of clinical disease. Due to the common subclinical infection status of SI in individual pigs (Er, Lium et al. 2014, Er, Skjerve et al. 2016), visual inspection could be ineffective in detecting disease in introduced pigs. About 6% of the visited farms purchased pigs from traders (middle men) or from live pig markets, where pigs from different farms are mixed. Mixing of pigs and contact of pigs from different sources can facilitate SI spread (Bowman, Nelson et al. 2014, Bowman, Workman et al. 2014, Lauterbach, Wright et al. 2018). Contact between pigs and infected buyers can be another risk factor for SI introduction (Grontvedt, Er et al. 2013, Nelson and Vincent 2015). Less than half of the surveyed farms sold the whole pen each time. Others have reported the association of SI infection with a lack of all-in all-out management in the fattening room (OR 2.4, 95% CI: 1.0–5.8) (Fablet, Simon et al. 2013). When selecting and loading pigs, on 30% of the farms buyers would participate in the activity. However, many of the surveyed farms did not ask the buyers to change their clothes (58%) or boots (72%) before entering the piggeries. Buyers sometimes purchase pigs from different farms to make up a consignment, with 11% of surveyed farmers reporting seeing trucks collecting their pigs already containing pigs from other farms. Since SIV can be transmitted through aerosols (Corzo, Romagosa et al. 2013, Hemmink, Morgan et al. 2016), the close proximity of pigs from other farms present on these trucks could introduce SIV via aerosols, or they could contaminate clothes or boots of people involved in the loading (Lauterbach, Wright et al. 2018).

Pigs can contract influenza A viruses from other species, especially from humans and birds (Karasin, Brown et al. 2000, Grontvedt, Er et al. 2013, Nelson and Vincent 2015). Avian influenza viruses have been isolated from pigs in many places. In Canada, H4N6 influenza A viruses were isolated from pigs with pneumonia on a commercial swine farm (Karasin, Brown et al. 2000). Human source influenza A infection in pigs has also been widely reported. For example, a study in the Czech Republic reported that antibodies against human influenza virus isolated during the 1995 epidemic were present in the local pig population. It is possible that the human virus was introduced to the pig herds by infected animal attendants, in whom antibodies against this virus were also found (Pospisil, Lany et al. 2001). In China, former prevailing human H1N1 strains have been shown to be circulating in the pig population (Yu, Zhang et al. 2007, Yu, Zhou et al. 2009). The authors concluded that more than 40 outbreaks of human-origin H1N1 viruses in swine had been reported in the 5 years after H1N1pdm09 was first detected in humans (Nelson, Stratton et al. 2015).

South China, especially Guangdong Province, is considered an epicenter for influenza (Ninomiya, Takada et al. 2002). Understanding the complexity of the interface between pigs and other species, including humans, is key to understanding the ecology of influenza in this area. The high proportion of farms with other species on farm, including cats/dogs (75%) and poultry (57%) in this study, can provide opportunities for potential cross-species transmission of influenza within this area. Similar to the findings of this study, in a small study (85 farms) conducted in Spain by Simon-Grife, Martin-Valls et al. (2011) the presence of other species on a farm increased the risk of infection with SI in fattening pigs (OR = 2.3). In contrast, Takemae, Shobugawa et al. (2016) found that the presence of other animals on a farm was protective for influenza A infection in pig farms in Vietnam (OR = 0.26). The conflicting results may be due to different ecosystems and husbandry practices adopted between studies. In the current study the presence of backyard poultry increased the risk of farmer-reported SI. Our survey found that 69% of farms with poultry introduced live poultry from local live bird markets, where high prevalences of avian influenza have been reported (Yuan, Lau et al. 2015). Compared to H5 and H7 subtype avian influenza, H9 subtype is the most common avian-sourced influenza infection in pigs. In China, 28 swine H9N2 viruses were isolated from 1998 to 2007 (Karasin, Brown et al. 2000, Yu, Zhou et al. 2011). Furthermore wild birds, particularly wild ducks, can be involved in the transmission of influenza viruses to pigs through contaminating pond water, and as SIV can also be transmitted to poultry, the possibility of SIV transmitting to wild birds cannot be ruled out (Karasin, Brown et al. 2000, Karasin, West et al. 2004, Kuntz-Simon and Madec 2009). Of the visited farms 89% had ponds, and 18% of them used the pond water to flush piggeries. Furthermore wild waterfowl are commonly seen on these ponds during the bird migratory seasons (personal communication with some interviewed pig farmers). Avian influenza virus can remain infective for more than 40 days in water at temperatures ≤ 23 °C, thus contaminated pond water could potentially introduce avian influenza virus to pigs through aerosolization during flushing (Lebarbenchon, Yang et al. 2011, Lebarbenchon, Sreevatsan et al. 2012).

The findings of this study can help reduce the risk of SI on pig farms and mitigate against the risk of a potential influenza pandemic. The study highlights the need for improved biosecurity in piggeries, particularly with respect to the introduction and sale of pigs. Local veterinary authorities should educate farmers on better biosecurity management to reduce the risk of SI and the findings from this study should be noticed in educational material for by local farmers. For example, farmers should follow an all-in all-out practice for batches/pens and should not let buyers enter piggeries. Farmers should particularly be aware that backyard poultry and wild birds on farm do have a potential negative impact to their pigs. As well as influenza virus, other pathogens, including Brachyspira pilosicoli and atrophic rhinitis pathogenic Pasteurella multocida (Dejong 1991, Smith 2005), can be transmitted from poultry to pigs. Active surveillance for SI is currently undertaken in south China by the National Reference Laboratory for Animal Influenza, and this is designed to monitor gene mutations of circulating SIVs and the early detection of new strains with potential pandemic threat (Chen, Zhang et al. 2013, Yang, Chen et al. 2016). To be more efficient, sampling should be conducted in early spring in Guangdong, and farms with poor biosecurity and particularly those with poultry, wild birds and other animals with access to the pigs should be specifically targeted for sampling.

3.5 Conclusions

This study has revealed several potential pathways for SI transmission among pig farms in Guangdong Province. Access by humans, poultry, wild birds and other animals on pig farms can increase the risk of SI infection in pig farms. The findings of this study highlight the importance of understanding the local pig industry and the practices adopted when developing control measures to reduce the risk of SI to local pig farms. It is concluded that biosecurity needs to be improved significantly to reduce the risks from SI in southern China.

3.6 Acknowledgements

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CHAPTER 4： Risk of Zoonotic Transmission of Swine Influenza at the Human-Pig Interface in Guangdong Province, China

Preface

In the preceding chapter, the interactions between pigs, chickens, dogs/cats and the pig farmers and the biosecurity practices adopted by the local farmers in Guangdong Province were described. Several potential pathways for the spread of SIV between and within local pig farms were identified. In particular there were frequent contacts between pig farmers and their pigs during routine management and husbandry practices and when pigs were sold. As influenza viruses can be transmitted from pigs to humans and vice versa, it would be useful to understand the risk of spill-over infection of viruses at the human-pig interface.

This chapter was designed to explore the knowledge, beliefs and practices of the pig farmers and live pig traders in Guangdong Province on SI. The practices adopted by local pig farmers and traders that would facilitate the spread of influenza between pig farms and between pigs and humans were described. In particular factors associated with a “low awareness of the zoonotic risk of SI” and “not using personal-protection equipment” were analysed to inform future targeted interventions against SI.

The text of this chapter is identical to that in the manuscript published in ‘***Zoonoses and Public Health***’ except for the reference list which has been combined with references of other chapters and incorporated as one list at the end of the thesis.

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<u>Principal Author</u>

Name of Principal Author (Candidate)	Yin Li
Contribution to the Paper	Conceptualised and developed the study, planned and conducted the field study, collected and analysed the data, interpreted the results and wrote the paper.
Overall percentage (%)	60
Signature	Date: 15/06/2020

<u>Co-Author Contributions</u>

By signing the Statement of Contribution, each author certifies that:

iii. the candidate’s stated contribution to the publication is accurate (as detailed above);
iv. permission is granted for the candidate to include the publication in the thesis.

Name of Co-Author	Emeritus Professor Ian Robertson
Contribution to the Paper	Supervised the study and provided critical comments to improve the interpretation of results, edited and revised the manuscript.
Overall percentage (%)	10
Signature	Date: 15/06/2020

Name of Co-Author	Emeritus Professor John Edwards
Contribution to the Paper	Provided critical comments to improve the interpretation of results, edited and revised the manuscript.
Overall percentage (%)	5
Signature	Date:15/06/2020
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Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	5
Signature	Date: 15/06/2020
Name of Co-Author	Dr Wang Youming
Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Professor Zhang Guihong
Contribution to the Paper	Collected data
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Dr Cai Chang
Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Dr Shen Chaojian
Contribution to the Paper	Conducted the field study and collected data
Overall percentage (%)	5
Signature	Date:15/06/2020

Abstract

A cross-sectional survey was conducted from 2015 to 2018 to assess the risk of zoonotic influenza to humans at the human-pig interface in Guangdong Province, south China. One hundred and fifty-three pig farmers, 21 pig-traders and 16 pig trade workers were recruited using convenience sampling and surveyed at local pig farms, live pig markets and slaughterhouses, respectively. Questionnaires were administered to collect information on the biosecurity and trading practices adopted and their knowledge and beliefs about swine influenza (SI). Most (12 of 16) trade workers said they would enter piggeries to collect pigs and only six of 11 said they were always asked to go through an on-farm disinfection procedure before entry. Only 33.7% of the interviewees believed that SI could infect humans, although pig farmers were more likely to believe this than traders and trade workers ($p < 0.01$). Several unsafe practices were reported by interviewees. “Having vaccination against seasonal flu” (OR = 3.05, 95%CI: 1.19 - 8.93), “Believe that SI can cause death in pigs” (No/Yes: OR = 8.69, 95%CI: 2.71 – 36.57; Not sure/Yes: OR = 4.46, 95%CI: 1.63 – 14.63) and “Keep on working when getting mild flu symptoms” (OR = 3.80, 95%CI: 1.38 – 11.46) were significantly and positively correlated to “lacking awareness of the zoonotic risk of SI”. “Lacking awareness of the zoonotic risk of SI” (OR = 3.19, 95%CI: 1.67 - 6.21), “Keep on working when getting mild flu symptoms” (OR = 3.59, 95%CI: 1.57 – 8.63) and “Don’t know SI as a pig disease” (OR = 3.48, 95%CI: 1.02 – 16.45) were significantly and positively correlated to “not using personal

protective equipment when contacting pigs”. The findings of this study would benefit risk mitigation against potential pandemic SI threats in the human-pig interface in China.

4.1 Introduction

Swine influenza not only causes significant economic loss to the pig industry (Er, Lium et al. 2014, Er, Skjerve et al. 2016), but is also a zoonosis that may cause serious public health problems worldwide (Dorjee, Revie et al. 2016). Patients infected by swine influenza strains show clinical signs of coughing, fever and running nose, similar to the signs of human influenza (Tang, Shetty et al. 2010). As with human seasonal influenza, deaths are occasionally reported in cases that have contracted swine strains (Bidiga, Asztalos et al. 2010, Gong and Gao 2010, Lee, Wu et al. 2010).

Cross-species transmission of influenza occurs because the influenza virus has segmented RNA, and gene exchange can happen when different strains infect the same host cells (Zhou, Senne et al. 1999, Kuntz-Simon and Madec 2009). Swine influenza reassortants have the potential to result in the next pandemic influenza of humans. The most recently known pandemic of swine-originated influenza in the human population were due to H1N1pdm in 2009. This virus had the capacity for rapid human to human transmission and contained genes from swine, poultry and human influenza strains (Schnitzler and Schnitzler 2009). Swine influenza is endemic in the Chinese pig population and the main virus strains circulating in the Chinese pig population include Eurasian avian-like H1N1, H1N1pdm, classical swine H1N1 and H3N2 (Chen, Zhang et al. 2013, Chen, Fu et al. 2014). New emerging strains make

China a country with a significant risk of producing new pandemic influenza strains (Chen, Zhang et al. 2013).

Pig farmers and traders in China have been found to have a higher risk of getting infected by swine/poultry influenza viruses (Zhou, Cao et al. 2014, Ma, Anderson et al. 2015) than people who don’t work in the livestock industry. Contacts between pig farmers, pigs, birds and dogs/cats have been highlighted in a recently published study (Li, Edwards et al. 2019) with 93% of respondents saying that workers lived on-site at the piggery with 29% of farms providing accommodation for staff immediately adjacent to pig houses. However, contacts between pig industry workers and pigs have been rarely studied and the knowledge and beliefs of Chinese pig industry workers about SI are not known.

A cross-sectional survey was undertaken to investigate human-pig contacts by pig farmers and live pig traders in Guangdong Province, China. The contacts between traders, trade workers and pigs were described to better understand the zoonotic risk of swine influenza in the trading sector in China. Gaps in the knowledge and beliefs of local pig farmers, traders and trade workers on SI were explored to inform targeted interventions in the future. These findings could benefit the mitigation of the risks of a potential pandemic influenza threat in China.

4.2 Materials and methods

4.2.1 Sampling strategy

The study was conducted in Guangdong Province, China. The interviews with pig farmers were undertaken in July and August 2015 and the meetings with pig traders and employees of pig traders were undertaken in June 2018. The sampling strategy used for selecting pig farmers was described in detail in a previous study (Li, Edwards et al. 2019). In brief, client lists of consultants who were offering veterinary services to pig farms in the province were used as a sampling frame, and on average seven farms were randomly selected from each of the 21 prefectures in the province. In total, 153 pig farmers were visited and surveyed. A convenience sampling strategy was used to choose traders and employees of traders. Twelve traders were interviewed at two wholesale live pig markets: Jinkang market (10 traders interviewed) and Jiahe market (2 traders interviewed). Nine traders were also interviewed at three slaughterhouses: Xincheng slaughterhouse in Xinxing county (3 traders), Kongwangji slaughterhouse (5 traders) and Shiqiao slaughterhouse (1 trader) in Guangzhou city. Sixteen trade workers (employees of traders) were interviewed in Jinkang market (8 workers) and Kongwangji slaughterhouse (8 workers). The locations of the premises where interviews were conducted are shown in Figure 4.1.

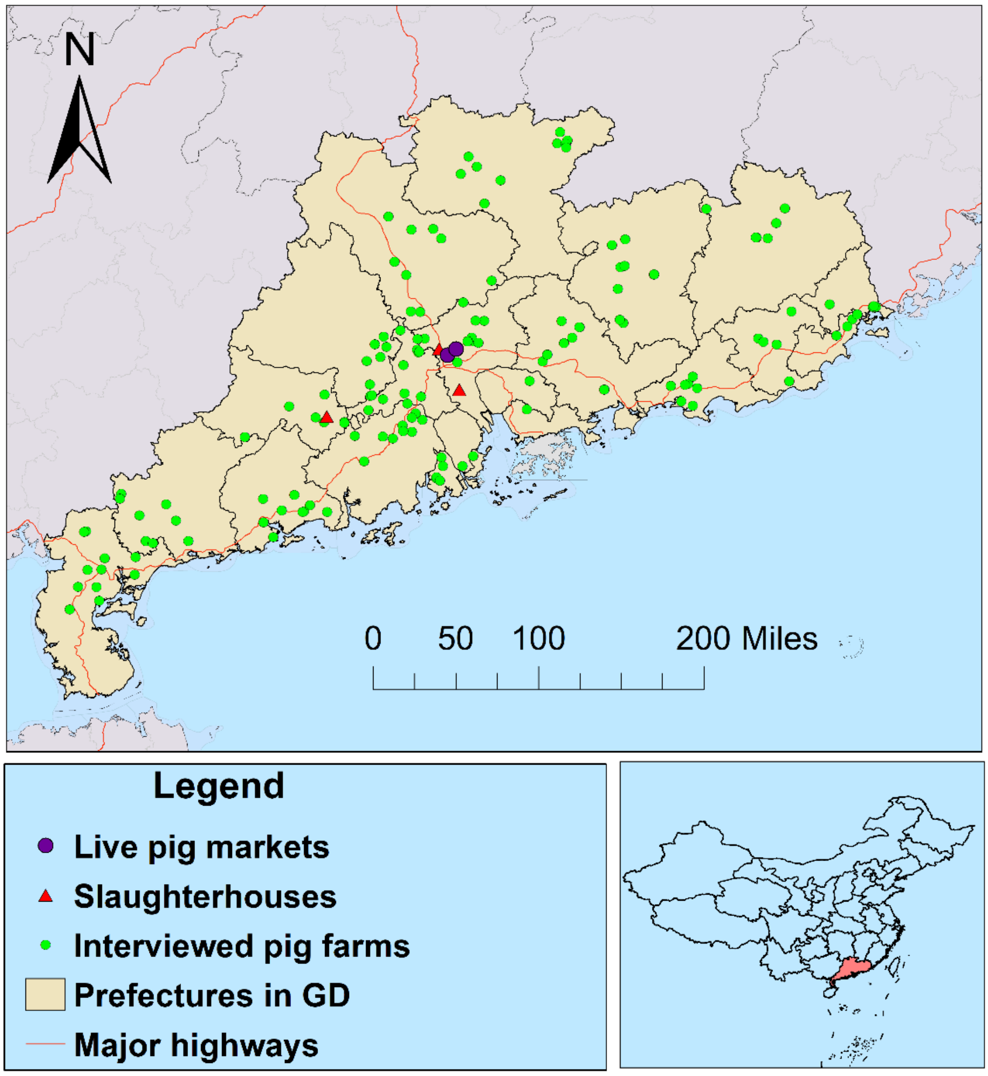

Figure 4.1 The location of the premises where interviews were conducted

4.2.2 Data collection

Questionnaires were designed and administered to collect information about human-pig contact, trade practices, and interviewees' knowledge and beliefs about SI and the practices they adopted in their daily work which may influence the risk of acquiring a zoonosis, such as SI. See appendix 1, 2 and 3. The farmers, traders and trade workers were asked different questions about contacts between themselves and their pigs because they played different roles within the pig industry. However, common issues on their knowledge, beliefs and practices that could increase the risk of SI were investigated across the three groups. To measure the knowledge and belief of interviewees, questions, such as, “Do you think SI can kill pigs” and “Do you think SI can infect humans" were asked. In terms of risky practices in their daily work, questions addressing the use of personal protection equipment (PPE), seeking of medications for influenza infection, and what they did when they got mild flu-like symptoms and SI infected pigs were asked. To avoid compromising the business reputation of the traders and trade workers, the question “How would your peers deal with pigs that are reluctant to walk during trade?” was asked to traders and trade workers, because the pigs infected by SI or other diseases would lay on the ground and not move unless forced to (Pomorska-Mol, Dors et al. 2017, Takemae, Tsunekuni et al. 2018). The questionnaires were pretested and subsequently revised. The final questionnaire for farmers, traders and trade workers contained 84, 25 and 15 questions, respectively and the average response time to complete was less than 30 minutes. Part of the questionnaire to farmers (30 questions) was analyzed in this study and the remaining questions were included in a separate study on prevalence, distribution and risk factors for farmer- perceived-SI-infection. The questionnaires were administered in a face-to-face setting. The interviewers were trained in delivering the questionnaire by the authors before administering the survey. The study and questionnaires were approved by the Murdoch University Human Ethics Committee [Project Number: 2017/113].

4.2.3 Data analyses

The contacts between local pig farmers and their pigs have been reported in another study and were not repeated in this study (Li, Edwards et al. 2019). Trade practices of traders and trade workers were analyzed using R software (version 3.0.2) (R Core Team 2018). Practices adopted by traders interviewed in markets and slaughterhouses were compared to explore different patterns in trade behaviors between the groups. Traders in live pig markets often collect pigs from intensively managed pig farms and then sell pigs to pork sellers (Li, Huang et al.), while traders in the slaughterhouses collected pigs from local backyard or small-scale farms. For different groups of traders/trade workers (working in markets vs. working in slaughterhouses), the percentages of people adopting different trade practices were calculated. The percentage was calculated using the number of people within a category divided by the total number of people of that group, compared to explore different patterns in trade behaviors between the groups. Practices adopted by interviewed pig farmers, traders and trade workers which could increase the risk of human acquired infections from SI, and knowledge and beliefs of interviewees about SI were compared. Similarly, the percentages of people undertaking different practices were calculated and compared between the groups. The chi-square test and t-test were conducted, when needed, using "Publish" package (Ozenne 2018) in R. Statistical descriptions of the knowledge, beliefs and unsafe practices of the interviewees were conducted and the differences between pig farmers, traders and trade workers were compared with the “Publish” package in R.

Factors associated with lacking awareness of the zoonotic risk of SI and not using PPE were explored with the “stats” package (R Core Team 2018) in R. An interviewee who didn't think or didn't know SI could infect humans was defined as a person who lacked awareness of the zoonotic risk of SI. An interviewee who didn't wear gloves or a face mask when having contact with pigs was defined as a person not using PPE in direct contact with pigs. Univariable and multivariable logistic regression analyses were used to identify the magnitude of the correlation between factors and interested outcomes. The odds ratio (OR) of each variable was calculated in the models, which reflect the magnitude of the association between the variable and the outcome of interest. The Hosmer–Lemeshow (HL) test and calculation of the area under the curve (AUC) were conducted to check the robustness of the multivariable logistic regression models. The packages “knitr” (Xie 2019) and “markdown” (JJ Allaire 2018) were used to produce the result tables.

4.3 Results

4.3.1 Trading behavior of traders and employees

The trading patterns of traders interviewed in slaughterhouses and markets are described in Table 4.1. Unsold pigs were kept and fed for additional days and traders interviewed in slaughterhouses were more likely to have leftover pigs than traders in markets ($p = 0.05$). Mixing pigs from different farms to make a batch to trade was a common practice with an average of pigs from 1.6 farms making up a trade (median 1). It took an average of 15 hours for a trader to sell all the pigs in one batch.

Table 4.1 Trade patterns of the interviewed traders in Guangdong province†.

Variable	Level	Location of interviewed traders		Total (n=21)	p-value
		slaughterhouse (n=9)	live pig market (n=12)		
Type of pigs traded	Finishers only	9 (100.0)	10 (83.3)	19 (90.5)	0.592
	Finishers and weaners	0 (0.0)	2 (16.7)	2 (9.5)	
Sizes of the farms supplying the trader (head)	< 100	1 (11.1)	1 (8.3)	2 (9.5)	0.489
	100-500	2 (22.2)	1 (8.3)	3 (14.3)	
	>500	3 (33.3)	2 (16.7)	5 (23.8)	
	Any size	3 (33.3)	8 (66.7)	11 (52.4)	
What is done with leftover pigs?	No leftover pigs	2 (22.2)	9 (75.0)	11 (52.4)	0.051
	Fed for a few days until selling	7 (77.8)	3 (25.0)	10 (47.6)	

Variable	Level	Location of interviewed traders		Total (n=21)	p-value
		slaughterhouse (n=9)	live pig market (n=12)		
Where do you purchase pigs from?	Contact pig farms directly	2 (22.2)	1 (8.3)	3 (14.3)	0.587
	Contracted pig farms	2 (22.2)	1 (8.3)	3 (14.3)	
	Middlemen	3 (33.3)	6 (50.0)	9 (42.9)	
	Pig farms owned by traders	1 (11.1)	0 (0.0)	1 (4.8)	
	Contact pig farms themselves & middlemen	1 (11.1)	2 (16.7)	3 (14.3)	
	Contact pig farms themselves & contracted farms & middlemen	0 (0.0)	1 (8.3)	1 (4.8)	
	Contact pig farms themselves & middlemen	0 (0.0)	1 (8.3)	1 (4.8)	
Where do you sell pigs to?	Sell live pigs to slaughterhouses	0 (0.0)	1 (8.3)	1 (4.8)	<0.01
	Slaughtered by slaughterhouses and then sell meat themselves	8 (88.9)	0 (0.0)	8 (38.1)	
	Sell live pigs to meat sellers	0 (0.0)	8 (66.7)	8 (38.1)	
	Sell live pigs to other live pig traders	0 (0.0)	1 (8.3)	1 (4.8)	
	Sell to slaughterhouses or meat seller	0 (0.0)	2 (16.7)	2 (9.5)	
	Slaughtered by slaughterhouse and then sell meat themselves and also sell pigs to other meat sellers	1 (11.1)	0 (0.0)	1 (4.8)	

Variable	Level	Location of interviewed traders		Total (n=21)	p-value
		slaughterhouse (n=9)	live pig market (n=12)		
Do you have another occupation?	No	4 (44.4)	12 (100.0)	16 (76.2)	0.013
	Meat seller	4 (44.4)	0 (0.0)	4 (19.0)	
	Meat seller & pig farmer	1 (11.1)	0 (0.0)	1 (4.8)	
Trucks for transport	Self-owned	7 (77.8)	6 (50.0)	13 (61.9)	0.399
	Rented	2 (22.2)	6 (50.0)	8 (38.1)	
How many farms are needed to make up a saleable batch	Median [iqr]	2.0 [1.0, 2.0]	1.0 [1.0, 2.0]	1.0 [1.0, 2.0]	0.582
Work experience in the pig industry (years)	Mean (sd)	10.0 (8.0)	10.5 (4.4)	10.2 (6.1)	0.872
On how many days would you visit at least one farm?	Mean (sd)	0.9 (0.2)	3.6 (8.3)	2.5 (6.5)	0.369
How many farms have you visited in the preceding 30 days?	Mean (sd)	2.9 (1.6)	13.1 (9.6)	9.0 (9.0)	<0.01

Variable	Level	Location of interviewed traders		Total (n=21)	p-value
		slaughterhouse (n=9)	live pig market (n=12)		
How many batches of pigs are transported each month?	Mean (sd)	43.3 (40.0)	28.3 (2.1)	34.8 (26.5)	0.191
How many pigs are in a batch? (head)	Mean (sd)	75.1 (75.3)	92.5 (12.8)	85.0 (49.3)	0.428
How many hours are needed to sell one batch of pigs?	Mean (sd)	17.2 (13.0)	13.8 (2.7)	15.3 (8.6)	0.377
How many workers do you hire?	Mean (sd)	2.8 (1.8)	4.0 (2.1)	3.5 (2.1)	0.175
What is the daily salary for a worker (RMB)?	Mean (sd)	160.0 (56.6)	241.7 (46.9)	230.0 (54.6)	0.025

†: The numbers in the table are the number of traders within each category of variables, unless defined specifically in the variable column. Numbers in brackets are percentages (%).

The practices of the trade workers are summarized in Table 4.2. Most (12 of 16) trade workers said they would enter piggeries to collect pigs and only three said they wouldn't enter piggeries, with one failing to answer this question. Only 3 of 11 trade workers reported that they were not always asked by pig farmers to change their boots before entering piggeries and 2 out of 11 trade workers admitted they were not always asked to change their clothes before entering piggeries. Half of the interviewed trade workers also raised pigs or poultry at home (Table 4.2).

Table 4.2 Trade practices of the interviewed trade workers in Guangdong province†.

Variable	Level	Location of interviewed workers		Total (n=16)	p-value
		pig market (n=8)	slaughterhouse (n=8)		
Number of days worked each month	Mean (sd)	27.1 (2.4)	30.0 (0.0)	28.6 (2.2)	<0.01
Number of hours worked each day	Mean (sd)	8.0 (0.0)	9.2 (2.1)	8.6 (1.5)	0.085
How many farms do you visit each day?	Median [iqr]	1.0 [1.0, 1.2]	1.0 [1.0, 1.0]	1.0 [1.0, 1.0]	0.296
Number of batches transported each day	Median [iqr]	1.0 [1.0, 1.0]	2.0 [1.5, 2.0]	1.0 [1.0, 2.0]	0.023
Total number of pigs transported each day (head)	Mean (sd)	62.0 (17.3)	66.4 (38.4)	64.1 (28.0)	0.768
Whether enter piggery to collect pigs	No	0 (0.0)	3 (42.9)	3 (20.0)	0.155
	Yes	8 (100.0)	4 (57.1)	12 (80.0)	
Whether required to change boots before entering a piggery	No	1 (12.5)	0 (0.0)	1 (9.1)	0.037
	Yes	7 (87.5)	1 (33.3)	8 (72.7)	
	Sometimes	0 (0.0)	2 (66.7)	2 (18.2)	
Whether required to change clothes before entering a piggery	Yes	8 (100.0)	1 (33.3)	9 (81.8)	0.094
	Sometimes	0 (0.0)	2 (66.7)	2 (18.2)	
	No	0 (0.0)	0 (0.0)	0 (0.0)	

Variable	Level	Location of interviewed workers		Total (n=16)	p-value
		pig market (n=8)	slaughterhouse (n=8)		
Whether required to undergo any disinfection procedure before entering a piggery	No	0 (0.0)	1 (33.3)	1 (9.1)	0.051
	Yes	6 (75.0)	0 (0.0)	6 (54.5)	
	Sometimes	2 (25.0)	2 (66.7)	4 (36.4)	
Whether raise pigs or poultry at home	No	5 (62.5)	3 (37.5)	8 (50.0)	0.617
	Yes	3 (37.5)	5 (62.5)	8 (50.0)	
Whether co-workers raise pigs or poultry at home	No	5 (62.5)	3 (37.5)	8 (50.0)	0.558
	Yes	1 (12.5)	1 (12.5)	2 (12.5)	
	Not sure	2 (25.0)	4 (50.0)	6 (37.5)	

†: The numbers in the table are the number of traders within each category of variables, unless defined specifically in the variable column. Numbers in brackets are percentages (%).

4.3.2 Knowledge and beliefs of pig farmers, traders and trade workers about swine influenza

The knowledge and beliefs of the interviewees were evaluated through the responses to four questions (Table 4.3). More than 90% of the interviewees were aware that SI was a pig disease, with more farmers (92.8%) knowing about the disease than trade workers (68.8%). Only 33.7% of the interviewees thought that SI could infect humans, with more farmers than the other two groups believing this (36.6% in farmers vs 14.3% in traders and 31.2% in trade workers) ($p < 0.01$).

Table 4.3 **Knowledge and beliefs of interviewed pig farmers, traders and trade workers in swine influenza†.**

Variable	Level	employees of traders (n=16)	pig farmers (n=153)	pig traders (n=21)	Total (n=190)	p-value
Work experience (years)	Mean (sd)	10.9 (4.8)	8.2 (5.2)	10.2 (6.1)	8.6 (5.3)	0.047
Whether aware of the disease called swine influenza?	No	5 (31.2)	11 (7.2)	2 (9.5)	18 (9.5)	<0.01
	Yes	11 (68.8)	142 (92.8)	19 (90.5)	172 (90.5)	
Do you think SI is a significant disease in pigs?‡	Yes	-	85 (55.6)	6 (28.6)	91 (52.3)	0.016
	No	-	41 (26.8)	6 (28.6)	47 (27.0)	
	Don’t know	-	27 (17.6)	9 (42.9)	36 (20.7)	
Do you think SI can kill pigs?‡	Yes	-	106 (69.3)	3 (14.3)	109 (62.6)	<0.01
	No	-	20 (13.1)	10 (47.6)	30 (17.2)	
	Don’t know	-	27 (17.6)	8 (38.1)	35 (20.1)	
Do you think SI can infect humans?	Yes	5 (31.2)	56 (36.6)	3 (14.3)	64 (33.7)	<0.01
	No	2 (12.5)	40 (26.1)	14 (66.7)	56 (29.5)	
	Don’t know	9 (56.2)	57 (37.3)	4 (19.0)	70 (36.8)	

†: The numbers in the table are the number of traders within each category of variables, unless defined specifically in the variable column. Numbers in brackets are percentages (%).

‡: Trade workers were not asked these questions due to the extremely low response rate in the pre-test of the questionnaire to them.

4.3.3 Risky practices adopted by pig farmers, traders and trade workers that would promote zoonotic risk of SI

The practices that could enhance the zoonotic risk of SI are summarized in Table 4.4. Only 38.9% of the interviewees would always wear gloves/masks when they had contact with pigs for their work. Approximately two thirds (69.2%) of respondents would visit a doctor if they had influenza-like symptoms. However when affected by a mild influenza-like syndrome, 82.6% of them would continue working, with more farmers (87.6%) doing so than traders (61.9%) or trade workers (62.5%) ($p < 0.01$). Vaccination coverage of the surveyed group against seasonal human influenza was low with only 24.2% being vaccinated. When pigs displaying clinical signs similar to SI were seen, more than 60% of the participants' peers would reportedly continue to trade these pigs, while 25% would return the pigs to the source farms. When evidence of SI was observed on local pig farms, most farmers (94.8%) would treat the sick pigs.

Table 4.4 Practices adopted by interviewed pig farmers, traders and trade workers which could increase the risk of human acquired infections from SI†.

Variable	Level	Employees of the traders (n=16)	Pig farmers (n=153)	Pig traders (n=21)	Total (n=190)	p-value
Do you wear gloves/masks when you have contact with pigs in your work?	No	7 (43.8)	61 (39.9)	9 (42.9)	77 (40.5)	0.732
	Always	6 (37.5)	58 (37.9)	10 (47.6)	74 (38.9)	
	Sometimes	3 (18.8)	34 (22.2)	2 (9.5)	39 (20.5)	
What would you do if you got the “flu”?	Go to see a doctor	3 (27.3)	114 (74.5)	11 (52.4)	128 (69.2)	<0.01
	Take some pills	7 (63.6)	37 (24.2)	7 (33.3)	51 (27.6)	
	Just have a rest without any medical treatment	1 (9.1)	2 (1.3)	3 (14.3)	6 (3.2)	
Would you continue to work if you had a mild case of the flu?	No	6 (37.5)	19 (12.4)	8 (38.1)	33 (17.4)	<0.01
	Yes	10 (62.5)	134 (87.6)	13 (61.9)	157 (82.6)	
Are you vaccinated against seasonal flu each year?	No	12 (75.0)	117 (76.5)	15 (71.4)	144 (75.8)	0.877
	Yes	4 (25.0)	36 (23.5)	6 (28.6)	46 (24.2)	

Variable	Level	Employees of the traders (n=16)	Pig farmers (n=153)	Pig traders (n=21)	Total (n=190)	p-value
In your opinion how would your peers deal with stressed pigs that were reluctant to walk?	Sell at a lower price	4 (25.0)	-	6 (30.0)	10 (27.8)	0.098
	Return to the original farm	2 (12.5)	-	7 (35.0)	9 (25.0)	
	Emergency slaughter	6 (37.5)	-	6 (30.0)	12 (33.3)	
	Notify authorities for safe disposal	0 (0.0)	-	1 (5.0)	1 (2.8)	
	Sell after treatment using antibiotics	4 (25.0)	-	0 (0.0)	4 (11.1)	
How would you deal with any pigs that had influenza-like clinical signs ‡?	Don’t take action	-	6 (3.9)	-	6 (3.9)	-
	Sell	-	2 (1.3)	-	2 (1.3)	
	Treat	-	145 (94.8)	-	145 (94.8)	

†: The numbers in the table are the number of traders within each category of variables, unless defined specifically in the variable column. Numbers in brackets are percentages (%).

‡: only farmers were asked this question

4.3.4 Factors associated with a lack of awareness of the zoonotic risk of SI

The factors associated with a lack of awareness of the zoonotic potential of SI are displayed in Table 4.5 and Supplementary Table 4.1. “Being vaccinated against seasonal flu” (OR = 3.05, 95%CI: 1.19 - 8.93), “Don’t think SI can cause death in pigs” (OR = 8.69, 95%CI: 2.71 – 36.57), “Don’t know if SI can cause death in pigs” (OR = 4.46, 95%CI: 1.63 – 14.63) and “Keep on working when having mild flu symptoms” (OR = 3.80, 95%CI: 1.38 – 11.46) were found significantly positively correlated to “lacking awareness of the zoonotic risk of SI” in the multivariable logistic regression analysis (p-value: 0.66 for HL test on the multivariable logistic regression model, AUC: 0.74 (95% CI: 0.67-0.80)).

Table 4.5 Results of the multivariable logistic regression analysis for lacking awareness of the zoonotic potential of swine influenza

	β	Sig.	OR	95% CI for OR	
				Lower	Upper
Is vaccinated against seasonal flu each year	1.116	0.027	3.051	1.19	8.93
Don't think SI can cause death in pigs	2.163	0.001	8.694	2.71	36.57
Don't know if SI can cause death in pigs	1.495	0.007	4.460	1.63	14.63
Keep on working when get mild flu-like symptoms	1.335	0.012	3.798	1.38	11.46
Constant	-1.197				

4.3.5 Factors associated with not using PPE when contacting pigs

The factors associated with "not using PPE when contacting pigs" are summarized in Tables 4.6 and Supplementary Table 4.2. "Lacking awareness of the zoonotic risk of SI" (OR = 3.19, 95%CI: 1.67 - 6.21), "Keep on working when having mild flu-like symptoms" (OR = 3.59, 95%CI: 1.57 – 8.63) and "Don't know if SI is a pig disease" (OR = 3.48, 95%CI: 1.02 – 16.45) were significantly positively correlated to "not using PPE when contacting pigs" in the multivariable logistic regression analysis (p-value < 0.01 for HL test on the multivariable logistic regression model, AUC: 0.72 (95%% CI: 0.65-0.79)).

Table 4.6 Results of the analysis by multivariable logistic regression for not using PPE when contacting pigs

	β	SE	OR	95% CI for OR	
				Lower	Upper
Lack awareness of the zoonotic risk of SI	1.16	0.33	3.19	1.67	6.21
Keep on working when get mild flu-like symptoms	1.28	0.43	3.59	1.57	8.63
Don’t know SI is a pig disease	1.25	0.69	3.48	1.02	16.45
Constant	-1.44				

4.4 Discussion

South China has a complicated eco-system shared by large populations of humans, pigs and poultry, and it has been recognized as one of the most important areas for global influenza control (Trevennec, Cowling et al. 2011, Kong, Ye et al. 2014). Understanding the epidemiology, especially the zoonotic risk, of influenza in this area is the key to development of a control strategy. To our knowledge, this is the first study to explore the zoonotic risk of swine influenza at the human-pig interface in China.

Trade practices undertaken by local live pig traders can facilitate genetic reassortment among SI strains and encourage the emergence of new strains with pandemic potential in the field (Zhu, Zhou et al. 2011, Bowman, Nelson et al. 2014). Traders and trade workers frequently visit pig farms and they often enter piggeries as part of their business. Mixing pigs from different pig farms to make a batch for transport would expose pigs to different influenza strains. Exposed pigs can contract influenza and start to shed virus within one day of initial exposure (Corzo, Romagosa et al. 2013, Hemmink, Morgan et al. 2016). Weaner pigs traded in the live pig markets and the returned pigs with SI-like clinical signs are dangerous potential pathways to introduce new reassortants to pig farms (Lauterbach, Wright et al. 2018). To mitigate the influenza risk in the live pig trade sector, we suggest using new tools, such as social media software that can offer real-time video communication and examination of pigs remotely, to avoid direct contact between traders and their employees and piggeries when trading pigs. Evidence-based regulations on live pig trade should also be established in live pig markets and slaughterhouses. For example, traders shouldn’t be allowed to trade piglets and finishers at the same time; and mixing pigs from different farms should be prevented.

Low awareness of the zoonotic ability of SI was found among local pig farmers and trading personnel surveyed. Surprisingly people who had been vaccinated against human flu had lower levels of awareness of the zoonotic risk of SI than non-vaccinated individuals. This may be associated with vaccinated people being less likely to become infected with swine strains because the administered human vaccines against seasonal influenza may provide some cross-protection against swine strains (Solorzano, Ye et al. 2010). However, if a new pandemic strain emerges in the pig population, it is unlikely that vaccination against human influenza strains would protect people (Centers for Disease Control and Prevention 2019). There are also several factors influencing the decision of people to be vaccinated against human influenza, including: access to medical care; perceived risk of contracting human influenza; perceived severity of human influenza; concerns on potential side effects arising from vaccination against human influenza; highest level of education and income status (Parrish, Graves et al. 2015, Maurer 2016, Quinn, Jamison et al. 2017, Wang, Yue et al. 2018). It is possible that there is an unknown confounding factor for the apparent association between uptake of vaccination against human influenza and lower levels of awareness of the zoonotic risk of SI. It is significant that more than 80% of the interviewees would keep on working when they had mild influenza-like symptoms, even though they potentially could transmit human influenza viruses to pigs (Nelson and Vincent 2015). This behavior was also found to be associated with "lacking awareness about the zoonotic risk of SI". We suggest that an education campaign should be conducted in China to promote farmers' and traders' awareness of the zoonotic risk of SI. According to the findings in the study, pig farmers and people involved in the live pig trade should be informed that SI can infect people, and if they develop influenza, they can also transmit the disease to pigs. As traders were shown to have a lower level of knowledge than farmers in this study it is recommended that traders be specifically targeted in any education program. We suggest that routine training should be offered by local health authorities and information provided on leaflets for distribution to the local live pig markets and slaughterhouses.

The interviewees’ knowledge and beliefs about SI have an impact on whether they would use PPE when contacting pigs. According to this study, the interviewees who didn't know about SI and were not aware that SI could infect humans, were more likely not to use PPE. It is essential to educate pig industry workers about the epidemiology of SI, and especially the zoonotic character of this disease. Regulations should be established to require the wearing of PPE, such as wearing a face mask and gloves, when contacting sick pigs by all pig industry workers in China.

4.5 Acknowledgements

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4.6 Conflict of Interest

The authors declare no conflict of interest.

4.7 Supplementary Material

Supplementary Table 4.1 Results of the analyses by univariable logistic regression for lacking awareness about the zoonotic risk of Swine Influenza

Correlated factors	P-value	OR (95%CI)
Occupation (traders and trade workers vs. farmers)	0.088	2.09 (0.93, 5.19)
Were vaccinated against seasonal flu each year	0.052	2.16 (1.02, 4.91)
Don’t think SI is an important disease to pigs	0.108	1.85 (0.89, 4.00)
Don’t know if SI is an important disease to pigs	0.003	4.86 (1.86, 15.25)
Believe SI can cause death in pigs (No/Yes)	0.003	5.51 (1.98, 19.61)
Believe SI can cause death in pigs (Not sure/Yes)	0.002	5.08 (1.98, 15.79)
Keep on working when get mild flu-like symptoms	0.019	2.49 (1.16, 5.38)

Supplementary Table 4.2 Results of the analysis by univariable logistic regression for not using PPE when contacting pigs

Factors	P-value	OR (95%CI)
Lack awareness of the zoonotic risk of SI	<0.001	3.65 (1.96, 6.94)
Don’t know SI is a pig disease	0.054	3.51 (1.02, 4.91)
Don’t think SI is an important disease to pigs	0.020	2.50 (1.17, 5.59)
Don’t know if SI is an important disease of pigs	0.458	1.35 (0.62, 3.00)
Keep on working when get mild flu-like symptoms	<0.001	4.04 (1.86, 9.25)

# CHAPTER 5:	Pig trade networks through live pig markets in Guangdong Province, China

Preface

Poor biosecurity and the frequent selling of pigs were found in the pig farms surveyed in Guangdong Province (Chapter Three). In addition the findings presented in Chapter Four highlighted that the trading practices of the local pig industry workers, especially the live pig traders, could promote the spread of influenza between pig farms in Guangdong Province. Mixing of pigs sourced from different pig farms at the local live pig markets can potentially result in the production of new SIV reassortants, with these new SIVs subsequently spreading via contaminated clothing of the traders and their vehicles when pigs are collected from pig farms. There are several wholesale live pig markets in Guangdong Province where, not only pigs from the province are traded, but also pigs sourced from other provinces are sold. The study reported in this chapter was developed to investigate the contact network between source counties that were connected to the wholesale live pig markets in Guangdong Province. Characteristics of the trading network were explored, and the impact of the structure of the network on controlling a potential epidemic was investigated with specific emphasis on the effectiveness of applying targeted (risk-based) control interventions for pigs sourced from counties with high levels of connectedness through the live pig market network.

The text of this chapter is identical to that in the manuscript published in '***Transboundary Emerging Diseases***' except for the reference list which has been combined with references of other chapters and incorporated as one list at the end of the thesis.

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Contribution to the Paper	Conceptualised and developed the study, planned and conducted the field study, collected and analysed the data, interpreted the results and wrote the paper.
Overall percentage (%)	60
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Co-Author Contributions

By signing the Statement of Contribution, each author certifies that:

v. the candidate’s stated contribution to the publication is accurate (as detailed above);
vi. permission is granted for the candidate to include the publication in the thesis.

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Overall percentage (%)	10
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Abstract

This study used social network analysis to investigate the indirect contact network between counties through the movement of live pigs through four wholesale live pig markets in Guangdong Province, China. All 14,118 trade records for January and June 2016 were collected from the markets and the patterns of pig trade in these markets analysed. Maps were developed to show the movement pathways. Evaluating the network between source counties was the primary objective of this study. A 1-mode network was developed. Characteristics of the trading network were explored and the degree, betweenness and closeness were calculated for each source county. Models were developed to compare the impacts of different disease control strategies on the potential magnitude of an epidemic spreading through this network. The results show that pigs from 151 counties were delivered to the four wholesale live pig markets in January and/or June 2016. More batches (truckloads of pigs sourced from one or more piggeries) were traded in these markets in January (8,001) than in June 2016 (6,117). The pigs were predominantly sourced from counties inside Guangdong Province (90%), along with counties in Hunan, Guangxi, Jiangxi, Fujian and Henan provinces. The major source counties (46 in total) contributed 94% of the total batches during the two-month study period. Pigs were sourced from piggeries located 10 to 1,417 km from the markets. The distribution of the nodes' degrees in both January and June indicate a free-scale network property, and the network in January had a higher clustering coefficient (0.54 vs 0.39) and a shorter average pathway length (1.91 vs

2.06) than that in June. The most connected counties of the network were in the central, northern and western regions of Guangdong Province. Compared with randomly removing counties from the network, eliminating counties with higher betweenness, degree or closeness, resulted in a greater reduction of the magnitude of a potential epidemic. The findings of this study can be used to inform targeted control interventions for disease spread through this live pig market trade network in south China.

5.1 Introduction

Live animal movement is a critical pathway for disease spread between farms, regions and countries (Bigras-Poulin, Thompson et al. 2006, Ortiz-Pelaez, Pfeiffer et al. 2006, Soares Magalhaes, Ortiz-Pelaez et al. 2010, Volkova, Howey et al. 2010). Understanding these movements is a key component of disease prevention and control. Social network analysis (SNA) have been utilized to: investigate the potential for disease transmission through animal movements; determine the magnitude and control of potential epidemics (Dube, Ribble et al. 2009, Gates and Woolhouse 2015); predict the infection risk for premises (Bigras-Poulin, Thompson et al. 2006); and guide risk-based surveillance approaches and decisions (e.g. early detection) (Kiss, Green et al. 2006, Martin, Zhou et al. 2011). Besides movement of live animals, attention has also focused on the network of indirect contacts between farms (Brennan, Kemp et al. 2008, Dent, Kao et al. 2008, Rossi, De Leo et al. 2017), because many animal diseases, including swine influenza (SI) and African swine fever (ASF), can spread indirectly via contaminated fomites (e.g. vehicles, equipment, clothing) and people (Grontvedt, Er et al. 2013, Lauterbach, Wright et al. 2018). A previous study in southern China highlighted the use of poor biosecurity practices by local pig farmers when selling pigs as: less than half of the farms implemented an “all-in-all-out” practice for pigs in a pen; thirty percent of buyers entered a piggery to select and collect pigs; and only about half of the surveyed farms always required all external vehicles to be disinfected (Li, Edwards et al. 2019). These behaviors in pig trade have the potential to facilitate the spread of contagious diseases via live pig trade networks.

Although pigs are usually transported directly from farms to slaughterhouses in most provinces of China, there is trade of live pigs through wholesale markets in Guangdong Province, in south China. Estimations have suggested that around 10% of the pigs slaughtered in the province, were traded through wholesale live pig markets (P. Chen, personal communication, July 10, 2018). Home slaughter of pigs is illegal in Guangdong Province and is rarely considered to occur in the field (People's Government of Guangdong People's Government of Guangdong Province 2011). Small abattoirs in townships offer a slaughter service at a cost of 30 RMB (4.5 USD) per head. Abattoirs with larger slaughter capacity are usually located in suburban areas of a city. All wholesale live pig markets in this province are located in the cities of Guangzhou and Foshan, and it is estimated that approximately 5.7 million pigs are supplied annually to these two cities via live pig markets (P. Chen, personal communication, July 10, 2018). Approximately 90% of the pigs traded at these markets originate from piggeries located within Guangdong Province (P. Chen, personal communication, July 10, 2018). In 2015, small piggeries, that sell less than 50 pigs in a year, contributed 87.5% of the total number of pig farms in Guangdong Province (Statistic Beurau of GuangdongStatistic Beurau of Guangdong Province 2016). The live pig traders or their employees usually visit pig farms in several counties every day to collect pigs for subsequent resale in the markets. These pigs are transported to the markets in trucks either owned or hired by the traders, and which usually carry pigs sourced from multiple piggeries. However, some pig farmers transport their pigs directly to the live pig markets. At the markets, the traders rent pens which are used to contain pigs purchased from multiple pig farms. The pens are separated from each other by either an open metal fence or a low brick wall (approximately 1 meter high). Pigs are then purchased by butchers/meat sellers. Some pig traders will offer a "slaughter and delivery service" where the pigs selected by the butchers are identified and sent to a slaughterhouse, with the carcass subsequently delivered directly to the meat seller's stall. The meat sellers’ stalls are not in the live pig trade markets and are often in a vegetable and meat market near residential areas. No pork is sold in these live pig trade markets. Pigs may stay in the markets for hours to days until being sent to slaughterhouses.

In 2016, the pig population in Guangdong Province was estimated at 20.5 million (Ministry of Agriculture and Rural Affairs 2017) and the province is considered a key area for the emergence of some important swine diseases in China. For example, the first case of FMD subtype A infection in a pig was found in the province in 2013 (OIE 2018) and in 2018, a novel coronavirus, swine acute diarrhea syndrome coronavirus, was identified as the pathogen causing high mortality in four commercial pig farms in the province (Zhou, Fan et al. 2018). There are also a variety of influenza strains circulating in pigs in the province and surveillance data indicates that the gene reassortment among local isolates is far more complicated than that among isolates from other Chinese provinces (Ninomiya, Takada et al. 2002, Liu, Wei et al. 2011, Cao, Zhu et al. 2013, Xie, Zhang et al. 2014, Zhou, Cao et al. 2014, Yang, Chen et al. 2016).

The live animal market trade system plays a critical role in the circulation of pathogens among areas in China, especially for long-distance disease spread (Martin, Zhou et al. 2011, Zhou, Li et al. 2015). However, live pig trade patterns in the markets have rarely been described and the characteristics of these networks and their impact on disease spread and control strategies to adopt have never been studied in China. This study was designed to investigate the indirect contact network between source counties through the movement of live pigs via these wholesale markets. This study aims to provide evidence for improved decision making and resource allocation to areas for prevention and control of disease. Trade patterns in live pig markets were described and properties of the networks in January (winter, busy trade season) and in June (summer, quiet trade season) were compared to study the stability of the live pig market trade network in different months/seasons. Different strategies were compared to illustrate the benefit of taking a risk-based intervention to constrain potential disease spread through this network. The findings of this study can be used to inform targeted interventions to control disease spread through the live pig market trade network.

5.2 Materials and methods

The objective of this study is to evaluate the trade network between source counties and the traders in wholesale live pig markets. This study was conducted in Guangdong Province, South China. SNA was used to explore the characteristics of this trade network. The trade data were collected by the China Animal Health and Epidemiology Center (CAHEC) during a routine survey in 2017. The study was approved by the Murdoch University Human Ethics Committee [Project Number: 2017/113].

5.2.1 Data sources

Trade records were extracted from health certificates of pigs and were collected from all four wholesale live pig markets in Guangdong Province, south China. In China, each batch of pigs requires a pig health certificate provided by the local official veterinarians. The pig health certificates are paper-based. The farmers give the pig health certificates to traders, so the traders can transport pigs to markets or slaughterhouses. If traders didn't offer pig health certificates, the markets or slaughterhouses will not accept their pigs (People's Government of Guangdong People's Government of Guangdong Province 2011). Market managers are required by local authorities to collect these health certificates and to keep them for at least one year. Three of these markets (Jiahe market - market 1; Furong market - market 2; and Baiyun market - market 3) are located in Guangzhou city, and Wufeng market (market 4) is situated in Foshan city. In total, 14,118 trade records from January

(8,001) and June (6,117) 2016 were collected. The data covers trade events from all traders in all four wholesale live pig markets. These markets are open every day of the year, except for a short closure (1-2 weeks) during the spring festival. Sheep, goat and cattle were also traded in Furong market, whilst all other markets only traded pigs. Data for each batch (a truckload of pigs that had been collected from one or more farms from the same county) was collected, including the source counties of the pigs (91.3% of the data had source counties recorded), the loading date, number of pigs, destination markets and the destination pig pen(s) at the market (76.1% of the data recorded the destination pen which is usually owned by one trader).

5.2.2 Data analyses

The patterns of pig trade in the four live pig markets were analyzed. Maps were developed using ArcGIS 9.3 (ESRI Inc., Redlands, CA, USA) to show the transport pathways from supply counties to the four markets and the average distance individual batches were transported was calculated. The total number of pigs traded in each month, the average size of a batch, the number of pig pens and the source counties of the pigs were also calculated for each market. A batch was a group of pigs from one or more farms transported to the live markets on one truck, irrespective of the number. A county that contributed at least 20 batches in one month was classified as a major supply county. The total number of batches to the markets from these major source counties was compared to check the stability of supply for January and June.

The SNA was conducted with the packages “igraph”(Nepusz 2006) and “tnet” (Tore Opsahl 2009) in R (R Core Team 2018). The study unit in the network was source county and trader. Firstly, the network was established as an undirected bipartite network, and the number of batches was set as the link weight. The source and destination nodes were set as the source counties and the pens (each pen owned by a trader) in the markets, respectively. The 2-mode network was then transformed into a 1-mode network by removing the pens, to focus on the network between source counties.

The static networks for markets 3 and 4, which had complete trade records, were compared between the two months (seasons) to evaluate the stability of the live pig trading networks through these markets. The 15 counties that contributed the most pigs to the two markets in January and June 2016 were compared to check the stability of the pig supply. The “power.law.fit” function in igraph was used to test if a network had free-scale property. The Kolmogorov-Smirnov test was used to test the goodness of fit for nodes with ten or more degrees using a confidence level of 95% (a p-value > 0.05 indicated that the nodes’ degree fit a power-law distribution, and thus the network has free-scale property).

Parameters (edge density, clustering coefficient, diameter, the average length of pathways) of the networks were calculated and compared (Nepusz 2006). Fast-greedy community detection was performed using the “fastgreedy.community” function in “igraph” to determine the number of communities in a network (Clauset, Newman et al. 2004). R0 was investigated across the networks. R0 is defined as the average number of secondary cases produced by a case during its infectious period in a susceptible population (Lin and Vandendriessche 1992). R0 is affected by the characteristics of the pathogen (for example, pathogenicity and environmental resistance of the pathogen). It is also determined by the method and frequency of contact between units of interest. In this study, we focused on the impact of the trader network on a disease transmitted among supply counties. To illustrate the impact of the network structure on the spread of diseases, we compared the R0s of existed networks in different seasons to simulate random networks with the same number of nodes. “R0(network)/R0(random)” was calculated for the two static networks in January and June 2016 (Marquetoux, Stevenson et al. 2016).

Trade data from January and June 2016 were joined to create a combined social network. The 2-mode network was then transformed into a 1-mode network by removing the pens (traders). There were 37 nodes deleted from the network because they were isolated nodes in the 1-mode network. These isolated counties infrequently supplied pigs to only one trader in the markets. The degree, betweenness and closeness of each node were calculated. The correlations between the nodes’ scores in degrees and betweenness and closeness were checked using Pearson’s correlation test. A map was developed to show the degrees of source counties in this network. The distributions of degrees, betweenness and closeness of the nodes in the combined network were illustrated with figures. To illustrate the impact of the key players on the potential magnitude of epidemics spreading through this network, the methodology of Marquetoux et al., (2016) was used to compare the decrease of the GWCC in the network with different strategies. One involved randomly removing a node in the network, while the others involved deleting the nodes in sequence according to their scores of three indicators of centrality: degree, betweenness and closeness.

Definitions of the technical terms used in this paper relating to SNA are provided in Table 5.1.

Table 5.1 Definitions of social network analysis terms used in the study on trade networks through live pig markets in Guangdong Province

Parameter	**Definition**
General terms:	
Node	A node refers to a unit of interest in a network (Dube, Ribble et al. 2009). In this study, supply counties and traders (sale pens in markets) are nodes in trade networks.
Edge	An edge represents a contact between individuals in the susceptible population (Shirley and Rushton 2005). In this study, counties were supplying pigs to a pen (2-mode network), or two counties were connected by the same trader(s). Links between a county and a pen (2-mode network) or between counties (1-mode network) were taken as an edge.

Parameter	**Definition**
Weight of links	In the bipartite network of counties and pens, the weight of a link was defined as the number of batches between a county and a pen, during a defined period. When projected as a 1-mode network of counties, the weight of a link was defined as the total number of paths (through pens) between two source counties, during a defined period.
Edge density	A value reflecting the density of the network and can be calculated using equation: L/k(k - 1). L means the number of exiting edges and k means the number of nodes in a network (Wasserman 1994)
Diameter	The longest geodesic between any pair of nodes in the network (Wasserman 1994)
Average path length	For any two given nodes, the shortest path between them over the paths between all pairs of nodes in the network (Dube, Ribble et al. 2009)
Measures of centrality:	
Degree	This parameter was calculated for the 1-mode network of source counties. It represents the total number of contacts of a county to other counties in the network. A higher degree means more connection to other nodes in the network (Marquetoux, Stevenson et al. 2016).

Parameter	Definition
Betweenness	The frequency by which a node falls between pairs of other nodes on the shortest path connecting them (Dube, Ribble et al. 2009). Betweenness is a measure of centrality used to quantify a node’s potential to ‘control’ the flow or curtail paths within a network (Marquetoux, Stevenson et al. 2016).
Closeness	The sum of the shortest distances (not geographical, but path length) from a source livestock operation to all other reachable operations in the network (Shirley and Rushton 2005)
Measures of cohesion:	
Clustering coefficient	This parameter was calculated for the 1-mode network of source counties. It represents the proportion of one county's neighbors who are also neighbors to another (Watts and Strogatz 1998).
Giant weakly connected component (GWCC)	The weakly connected component is the undirected subgraph in which all nodes are linked, not taking into account the direction of the links (Robinson and Christley 2007). GWCC is the largest weak component in the network (Dube, Ribble et al. 2009). In this study, the network among source counties was considered as an undirected network, so we use GWCC as the indicator for the potential magnitude of an epidemic.

5.3 Results

5.3.1 Trade patterns of the live pig market trade network

Pigs from 151 counties were delivered to the four markets in January and/or June 2016. There were at least 238 pens in operation in the four markets in these two months in 2016. On average 67 pigs were consigned in a batch. The daily trade volume in the four markets varied from 1,021 to 7,138 head (16 to 124 batches). Market 1 had the highest daily trade volume (5,954 and 7,138 pigs, and 77 and 124 batches in January and June, respectively). More batches were traded in the four markets in January (8,001) than in June 2016 (6,117). However, pigs were sourced from more counties in June (136) than in January 2016 (90) (Table 5.2).

Table 5.2 Trade statistics for the wholesale live pig markets in Guangdong in 2016

Market	Month	Number of batches	Total number of pigs	Average batch size ± SD	Number of recorded pig pens	Averaged daily trade volume (head) ± SD	Number of supply counties
1	January	3838	221293	58±33	96	7138±1506	65
1	June	2376	184577	78±24	4*	6153±3425	89
2	January	503	31638	63±33	1*	1021±121	22
2	June	491	34367	70±17	1*	1146±154	41
3	January	1515	126112	83±11	53	4068±282	38
3	June	1357	112175	83±17	51	3739±286	54
4	January	2145	125527	59±22	79	4049±661	36
4	June	1893	111196	59±23	85	3707±444	48
Total		14118	946885	67±24	-	-	151

*Most data in this month didn't include a record of the pen code. For the other unmarked numbers, the number of recorded pig pens was the number of pens in operation in that market during the respective month. The number of pens that a county was linked to during a month varied from 1 to 86. On average, pigs from a county were supplied to 12 (median: 5) pens in January and 8 (median: 2) pens in June 2016.

The sourcing counties were predominantly inside Guangdong Province (92% of all batches), along with counties in Hunan, Guangxi, Jiangxi, Fujian and Henan provinces (Fig. 5.1). The number of pigs supplied from different counties varied between January and June, but the supply from the major source counties was stable with the counties that contributed the most pigs/batches in January also providing the most in June (Fig.

5.2). The major source counties (46) contributed 94% of the total batches during the two months. Pigs were sourced from piggeries from 10 to 1,417 km from the markets, with average distances of 223 and 307 km in January and June 2016, respectively.

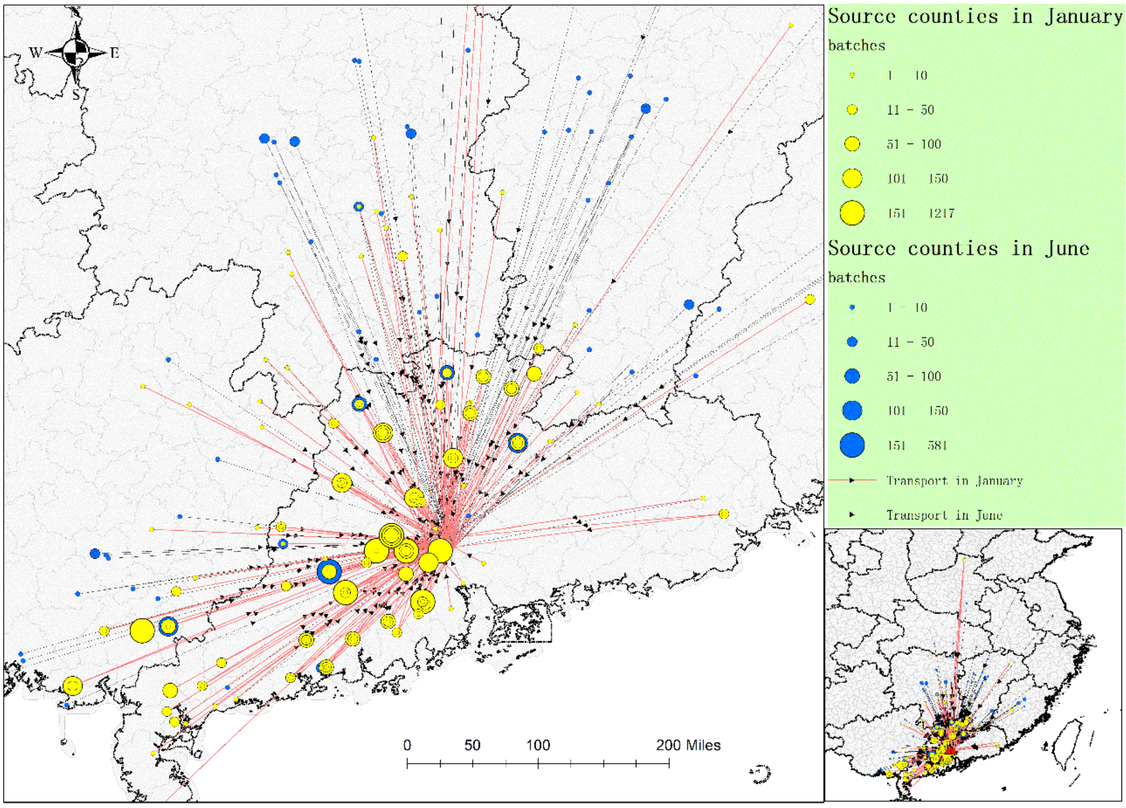

Figure 5.1 Transport of pigs to the wholesale live pig markets in Guangdong in January (high demand month) and June (low demand month) 2016. Yellow circles represent the source counties in January 2016 and blue circles represent the source counties in June 2016. Size of the circles indicates the number of batches transported. Circles overlapped for some counties because these counties supplied pigs to more than one market and each of the overlapping circles indicates the number of batches delivered to one of the markets.

Figure 5.2 Number of batches from major supply counties (supplying ≥ 20 batches/month) to the wholesale live pig markets in Guangdong in January and June 2016. Provinces of these counties are identified on the far right.

5.3.2 Trade networks in different months

The 2-mode trade network was analyzed to determine the stability of the trade between the two months/seasons. Twelve of the 15 counties supplying the most batches were similar in the two seasons. They contributed 84 and 78% of the total number of batches in January and June, respectively. Notably, all the links between the source counties in January still existed in June, with only 9% of the links in June being new links to the trade network; on the other hand, these new links only contributed 1.5% of the total number of batches in June.

The distributions of the degrees of the nodes in both January and June indicate free-scale network property (Fig. 5.3； p-values of 0.14 and 0.52, respectively), thus a few nodes have much higher connectivity than other nodes in this network. However, the network in January had a higher clustering coefficient and a shorter average pathway length than that in June (Table 5.3).

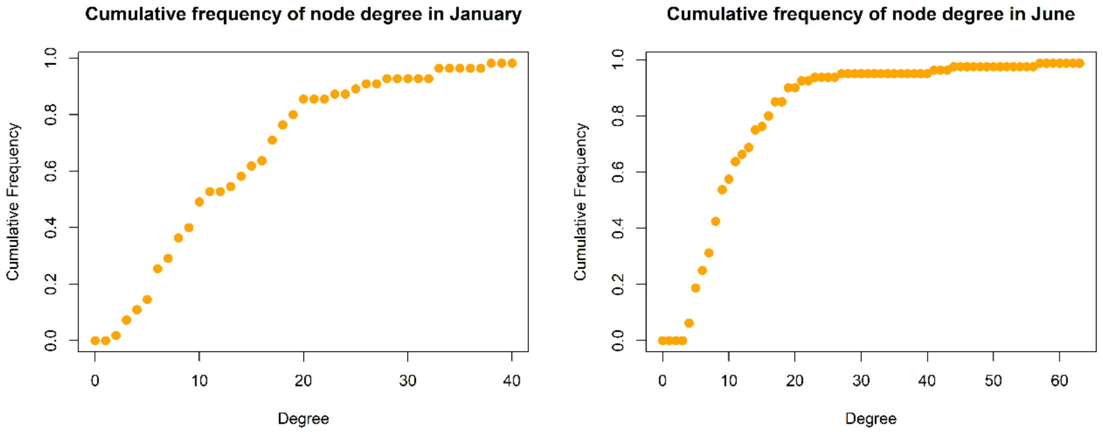

Figure 5.3 Distributions of the degrees of source counties in the live pig trade networks through wholesale live pig markets in Guangdong in January and June 2016

Table 5.3 Properties of pig trade networks through live pig markets in Guangdong Province in January and June 2016

Network properties	Month	
	January	June
Edge density	0.24	0.15
Clustering coefficient	0.54	0.39
Diameter	5	4
The average length of pathways	1.91	2.06
Number of communities	4	7
R0(network)/R0(random)	1.23	1.29

With 46 new source counties being added to the markets in June, new communities were formed in the live pig trade network (Supplementary Figure 5.1).

5.3.3 Properties of the combined static 1-mode network

The parameters of the combined static network are summarized in Table 5.4. Most of these parameters are between the parameters of the static networks of January and June. The combined static network is displayed in Fig 5.4.

Table 5.4 Properties of the combined (January and June) static social network of live pigs traded through live pig markets in Guangdong Province in 2016

Network properties	Value
Edge density	0.18
Clustering coefficient	0.47
Diameter	4
The average length of pathways	1.93
Number of communities	5
R0(network)/R0(random)	1.29

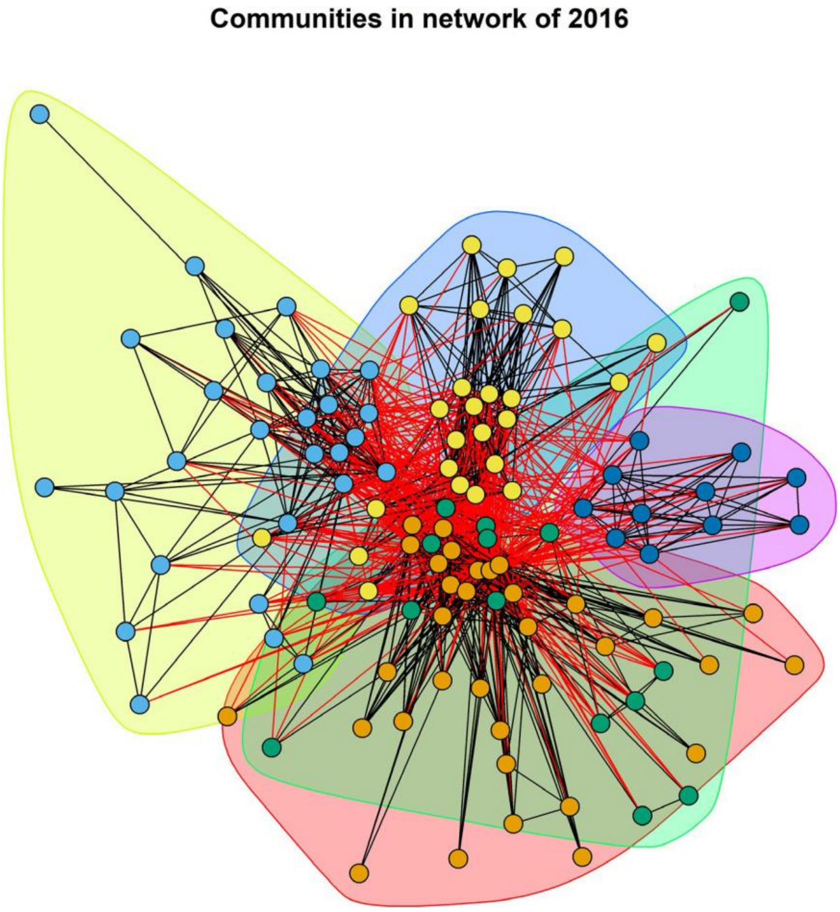

Figure 5.4 Graph of the combined static network of pig movement through wholesale live pig markets in Guangdong in January and June 2016. Different colored areas represent five different communities in the network, and nodes with the same color belong to the same community.

The degree, betweenness, and closeness of each source county in this network are summarized in Supplementary Table 5.1. The degree of each source county indicated that the most connected counties of the network were in the central, northern and western regions of Guangdong Province (Fig. 5.5).

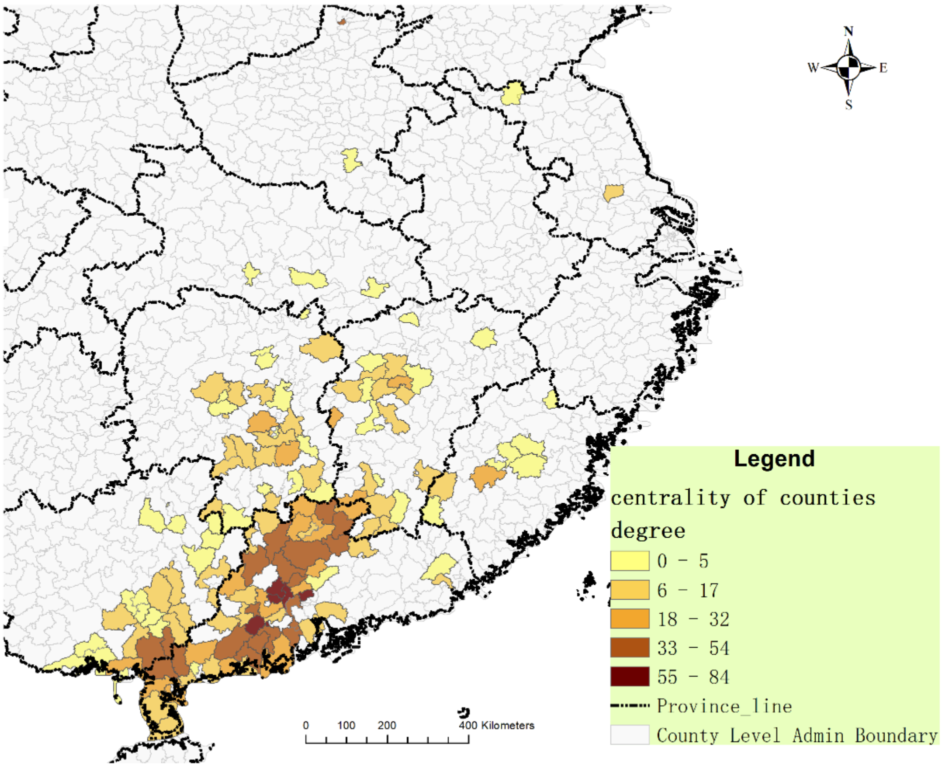

Figure 5.5 The connectivity of source counties in the combined static network of pig movement through wholesale live pig markets in Guangdong in January and June 2016.

5.3.4 Influence on GWCC by different 'control' strategies

The distribution of degree, betweenness and closeness are displayed in Supplementary Figure 5.2, 5.3 and 5.4. The nodes that had higher degrees also had higher betweenness (correlation coefficient of 0.88, $p < 0.001$) and higher closeness (correlation coefficient 0.74, $p < 0.001$). Compared with randomly removing counties from the network, eliminating counties with higher betweenness, degree or closeness, resulted in a greater reduction in the magnitude of a potential epidemic. Of the three risk-based strategies, isolating the nodes according to their betweenness had the greatest effect in decreasing the size of GWCC in most of the steps (Fig. 5.6).

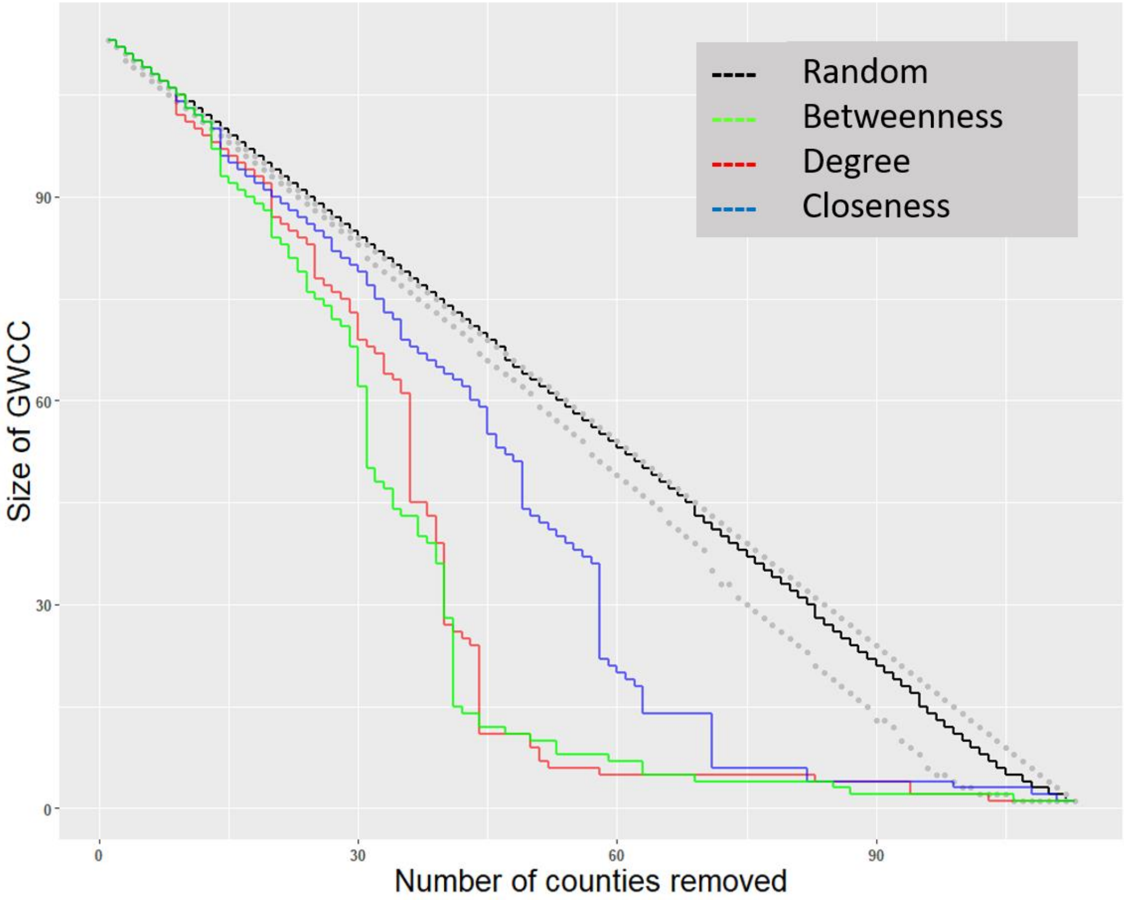

Figure 5.6 The decrease in the size of GWCC of the pig movement network through wholesale live pig markets in Guangdong in January and June 2016 under different control scenarios. The grey dotted lines representing the 95% CI of the size of the GWCC when removing counties randomly.

The GWCC reduced slowly when deleting the first few nodes with the highest degree, betweenness and closeness, and significant reductions only occurred when more nodes were deleted. For example, when less than 7 nodes were deleted from the network, there was no difference between the different strategies in terms of decreasing the size of the GWCC. However, if the 45 counties with the highest betweenness or degree from the network were removed, the GWCC decreased to approximately 10 counties, while if 45 counties were randomly removed, the GWCC decreased to only 65 counties (Fig. 5.6).

5.4 Discussion

To our knowledge, this is the first study that described the pattern and explored the network of live pig trading through wholesale live pig markets in China. Live animal markets provide a location where there is direct contact and mixing between animals and humans that can facilitate disease spread (Kiss, Green et al. 2006, Myers, Olsen et al. 2006, Robinson and Christley 2007, Bowman, Nelson et al. 2014, He, Liu et al. 2014, Zhou, Li et al. 2015, Dutkiewicz, Zajac et al. 2018, Van der Poel, Dalton et al. 2018). Furthermore, long-distance transport and mixing of animals at live-animal markets is stressful (Dalla Costa, Lopes et al. 2017, Earley, Buckham Sporer et al. 2017, Sommavilla, Faucitano et al. 2017, Zurbrigg, van Dreumel et al. 2017), allowing greater opportunity for pathogen and disease spread between animals.

Significant differences were found in the connectivity of source counties. “Free-scale” pattern was found in this market trading network. Studies conducted in other countries on livestock movement networks have also reported “free-scale” property (Woolhouse, Shaw et al. 2005, Kiss, Green et al. 2006, Lentz, Kasper et al. 2009, Soares Magalhaes, Ortiz-Pelaez et al. 2010, Molia, Boly et al. 2016, Earley, Buckham Sporer et al. 2017). This indicates that they are potentially key players in a network which should be targeted for disease control strategies. The connectivity was measured with different parameters in this study: degree, betweenness and closeness. Most of the counties with the highest degree were located in central, northern and western Guangdong. It is worth noting that the counties with high degree scores also had high betweenness and closeness values. These counties had larger pig populations than counties with lower connectivity. Thus, they are more likely to supply pigs to many markets at the same time. This result indicates that it will be very challenging to stop pig movement in counties with higher connectivity, during an emergency response to an epidemic. We suggest that besides movement suspension, other control measures such as emergency vaccination, enhanced quarantine, promotion of better biosecurity practices in the trading sector and education programs should also be implemented during emergency disease responses.

The results of this study have provided insight on approaches for implementation of emergency responses to SI and other pig diseases in Guangdong Province. For example, the transmission of ASF in China (Ge, Li et al. 2018, Normile 2018) has been the result of long-distance movement of live pigs allowing the epidemic to propagate (Wang, Sun et al. 2018). Our findings show that the supply counties of the live pig markets in Guangdong Province included, not only counties inside this province but also counties from Guangxi, Hunan, Jiangxi, Fujian, Jiangsu and Henan provinces. Animal health authorities in Guangdong Province should pay more attention to outbreaks in these provinces, especially Hunan and Guangxi. These provinces contributed more pigs to the live pig markets in Guangdong Province than did other provinces (excluding Guangdong Province) and some counties in the middle of Hunan and east Guangxi had relatively high connectivity in the live pig market trading network. For early detection of ASF in Guangdong Province, counties inside/adjacent to Guangzhou and to the north and south-west of the province should also be targeted for active surveillance. Control measures against ASF adopted in China have included mass field screening of pigs, widespread sampling and testing, movement restrictions, thorough cleaning and disinfection of trucks transporting pigs, and registration of live pig-traders (Ministry of Agriculture and Rural Affairs 2018). However, these measures can be a big burden for local governments. Social network analysis on animal movement can contribute to improving the efficiency of control measures when resources are limited by targeting priority areas. The current results indicate that, in an emergency response where often there are limited diagnostic and human resources, targeted surveillance and intervention would be a better strategy to control the potential magnitude of an outbreak among the source counties included in the market trading network. In this study, we found that if we isolated the 45 counties with higher connectivity (for example, by movement restriction or enhanced quarantine), the magnitude of a potential epidemic could decrease substantially, in contrast to conducting control measures across the same number of randomly selected counties (10 vs 70).

The findings of this study can also help to improve the efficiency of routine surveillance on influenza in this area. Influenza is one of the most significant zoonotic diseases (Myers, Olsen et al. 2006, Bowman, Nelson et al. 2014, Ma, Anderson et al. 2015, Lauterbach, Wright et al. 2018). Pigs can be infected by swine influenza strains, as well as some human strains, and genetic reassortment between swine and human influenza strains may facilitate the evolution of new strains circulating in pigs or even pandemic strains in humans (Zhou, Senne et al. 1999, Kuntz-Simon and Madec 2009, Rajao, Walia et al. 2017). A recent study indicated a poor level of biosecurity being adopted by pig farmers in Guangdong when selling pigs (Li, Edwards et al. 2019). There is evidence to indicate that workers on pig farms and markets in China have a higher risk of acquiring SI influenza than the general population (Yin, Rao et al. 2014, Ma, Anderson et al. 2015). To improve the efficiency of surveillance of SI in Guangdong, those traders in the markets with more contacts to different counties and those pig farms within the counties with higher connectivity in the network should be targeted for human influenza and SI surveillance, respectively.

The clustering coefficient was higher in the trade network of January than June 2016 (0.54 vs 0.39). Thus, via this market trading network, an epidemic in January would spread faster than in June. The average path length in the combined static network was less than 2, which means that any two counties in the network can be connected via just another county. Interestingly, the average path length was shorter in the trade network of January than that in June, which illustrates that it would be easier for a pathogen to spread among nodes in this trade network in January than in June. Furthermore, the lower temperature in January could preferentially influence the survival of pathogens in the environment (Botner and Belsham 2012). Local animal health authorities should be aware that this market trade network would require more attention in January.

The dynamics of the live pig trade can lead to new directions for pig diseases spread through this market trading network. We evaluated the consistency of the live pig market trading network by comparing the source counties in January and June 2016. The links among the dominant source counties and the live pig pens were stable in the different months, although there were 55 more counties from neighboring provinces involved in pig supply in June. However, these newly added counties contributed less than 2% of the pigs, and the trade frequency of these counties was low. When we transformed the 2-mode network into the 1-mode network, many of these counties became isolated nodes. We decided to simplify the network by deleting these nodes, because these counties which only occasionally supply pigs should have a low impact on the spread of disease between counties. It was not surprising that more pigs were traded in these markets in January than in June (504570 vs 442315) because January is close to the Chinese Spring Festival and demand for meat increases before this festival (Pan, Wei et al. 2016). The increase in the number of source counties in June may be due to the change in the pig density in Guangdong Province arising from a policy to restrict the number of pig farms in the province implemented in 2014, resulting in some farms being forced to close or to relocate to other counties (China State Council 2013). As displayed in Figure 5.2, Baiyun (suburban area of Guangzhou city) and Sanshui (suburban area of Zhongshan city) supplied many pigs in January 2016, but the numbers supplied in June 2016 decreased dramatically. During our field investigation, we were told that many pig farms in these areas were closed in 2016 because of concerns arising from their environmental impact. Another reason could be price changes in neighboring provinces which may have provided incentives for traders to collect pigs from more distant counties. A study on live poultry movement in China reported that when the price of poultry changed in neighboring provinces, the direction of movement of live poultry also changed accordingly (Li, Wang et al. 2018). It is worth noting that the increased supply counties in this network resulted in a change in the structure of the local market trade network. Newly produced communities can result in new disease circulating directions between counties. We suggest that local veterinary authorities should pay attention to the impact of policy or price changes on livestock movement. Monitoring the changes in the structure of this market trade network is needed.

Several model limitations need consideration. Firstly, the “removal of the counties from this network” cannot be totally achieved because illegal trade could be present. However, animal movement suspension has been implemented on several occasions in China (for example, emergency responses for PPR and ASF) (Ministry of Agriculture and Rural Affairs 2019) and is required by the animal health law in China (China State China State Council 2015). Although it is impossible to prevent all illegal trade, suspending legal trade would dramatically reduce the trade volume from selected counties. Besides movement restriction, other control measures, such as emergency vaccination, intensive screening for cases, enhancing biosecurity within the trading sector and implementing education programs could also reduce disease risk in targeted counties. These control measures could also result in the targeted areas being “removed” from the network in terms of spreading disease. Secondly, in reality authorities would not randomly select places to implement an emergency response. The places are usually selected according to their current infection status or potential for infection. However, early detection of an epidemic in a county can be challenging, especially for an exotic disease (Liu, Atim et al. 2019), and choosing counties based on convenience is unlikely to be effective. We used randomness to model these non-targeted scenarios and we believe that our model, even with its limitations, has offered new insights for decision-makers to understand the disease risk in places before an epidemic occurs. The same methodology has been used in another similar study (Marquetoux, Stevenson et al. 2016). It is worth noting that this study only focused on the pig movement network through local wholesale markets. Pigs are also traded through other systems in this province. For example, breeding pigs are often traded directly between pig farms, and weaners are often moved from breeding farms to fattening farms. Further studies on the movement of live pigs among local farms are needed.

It is a better strategy for disease control to understand the risk of disease spread through live animal movements before an epidemic actually occurs (Shirley and Rushton 2005). In recent years, many countries and companies have established databases to record livestock movement (Bigras-Poulin, Thompson et al. 2006, Kiss, Green et al. 2006, Marquetoux, Stevenson et al. 2016, Lee, Polson et al. 2017). These data would be critical in tracing livestock movement during emergency responses and would favor SNA being used to inform the establishment of a proper disease-control contingency plan. However, livestock trade (source, destination) is poorly recorded in many livestock markets in China. The most common source of live pig movement records in China is the official health certification of the traded pigs, which has limitations. Firstly, the certification record often lacks the name and location of the source farm, which makes it difficult, if not impossible, to effectively trace back to farms/animals in outbreak investigations. Secondly, data from the health certification system is not shared between provincial animal health authorities, even though live pig movements often cross provincial boundaries. A more comprehensive national database should be established in China. Detailed information on the location, species, farm size, type of source piggery (breeding, fattening etc.) and livestock movements should be recorded and updated in a timely manner.

5.5 Conclusions

The live pig market trading network in Guangdong involved pigs sourced from at least 151 counties in 2016. The trading network had connected counties in Guangdong, Guangxi, Hunan, Fujian, Jiangxi, Hubei, Henan and Jiangsu provinces. For emergency disease control, targeted surveillance is required, and for this to eventuate nation-wide, a more comprehensive database of livestock movement is needed at the national level. The findings in this study could be used to offer insights into SI surveillance, emergency responses and control of ASF and other swine diseases in Guangdong Province and southern China.

5.6 Acknowledgements

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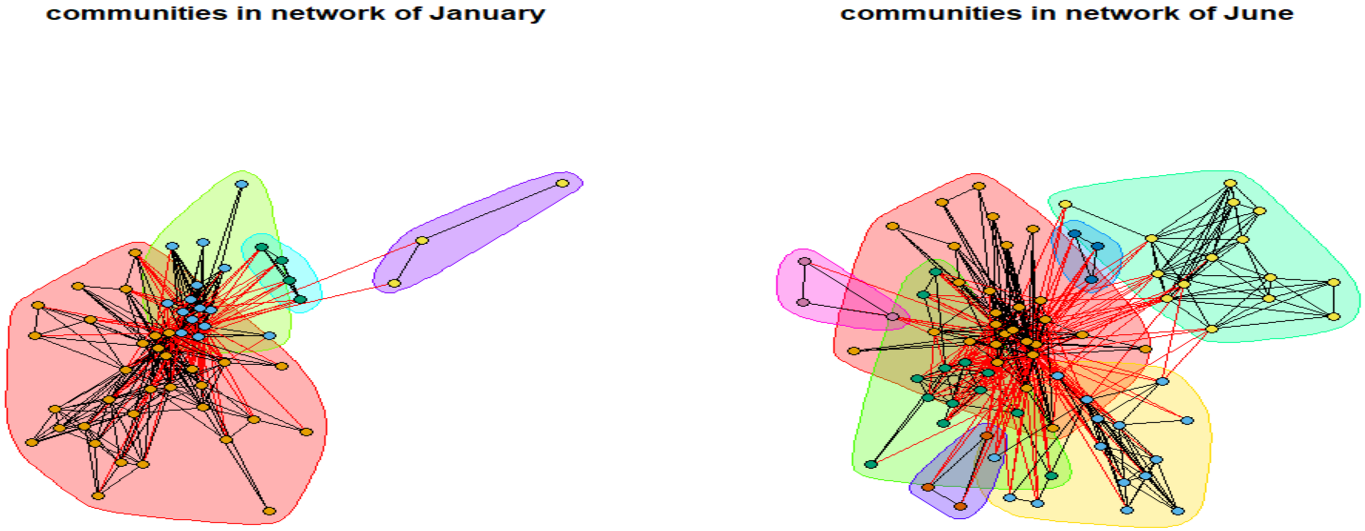
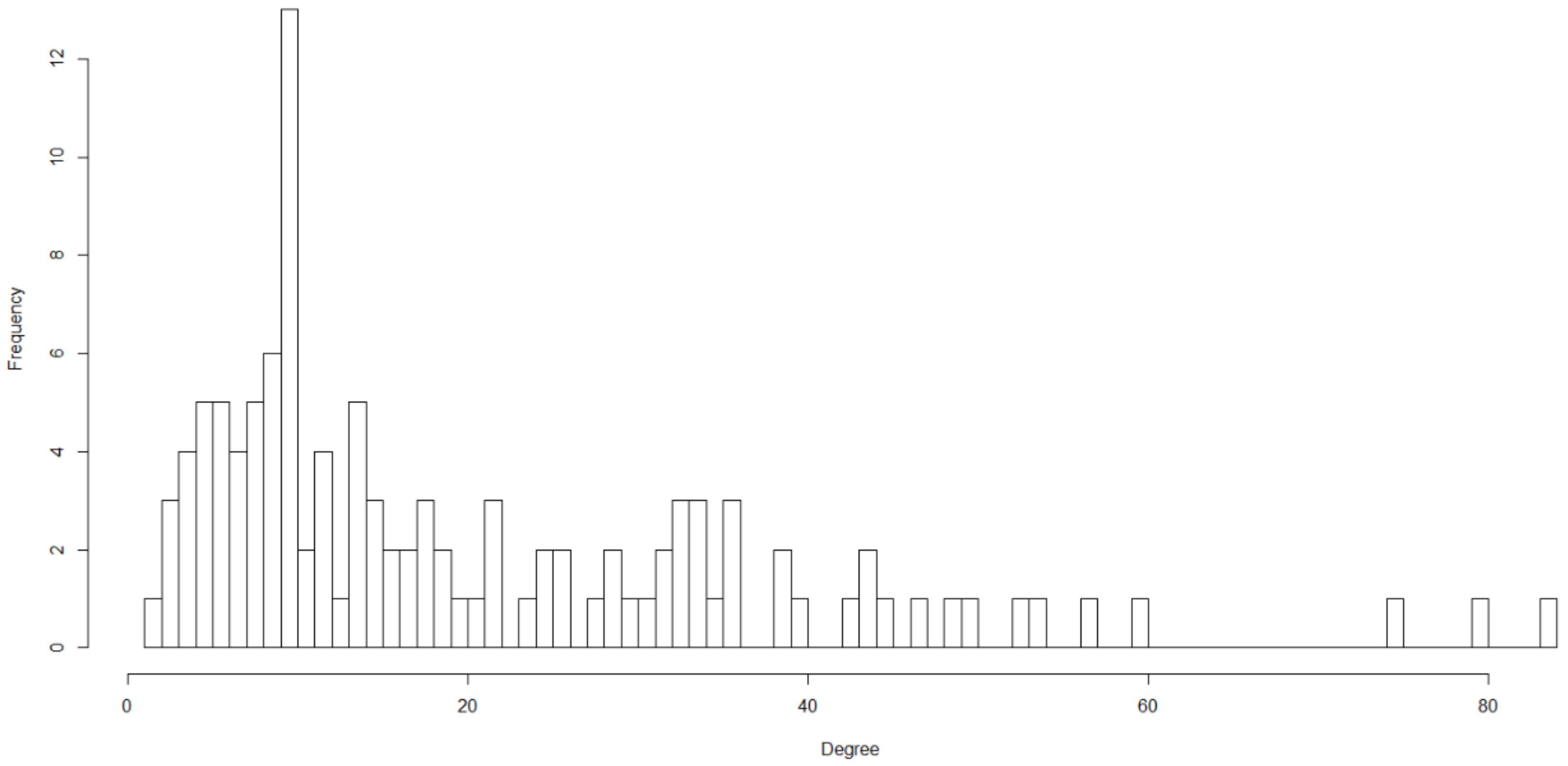
5.7 Supplementary Material

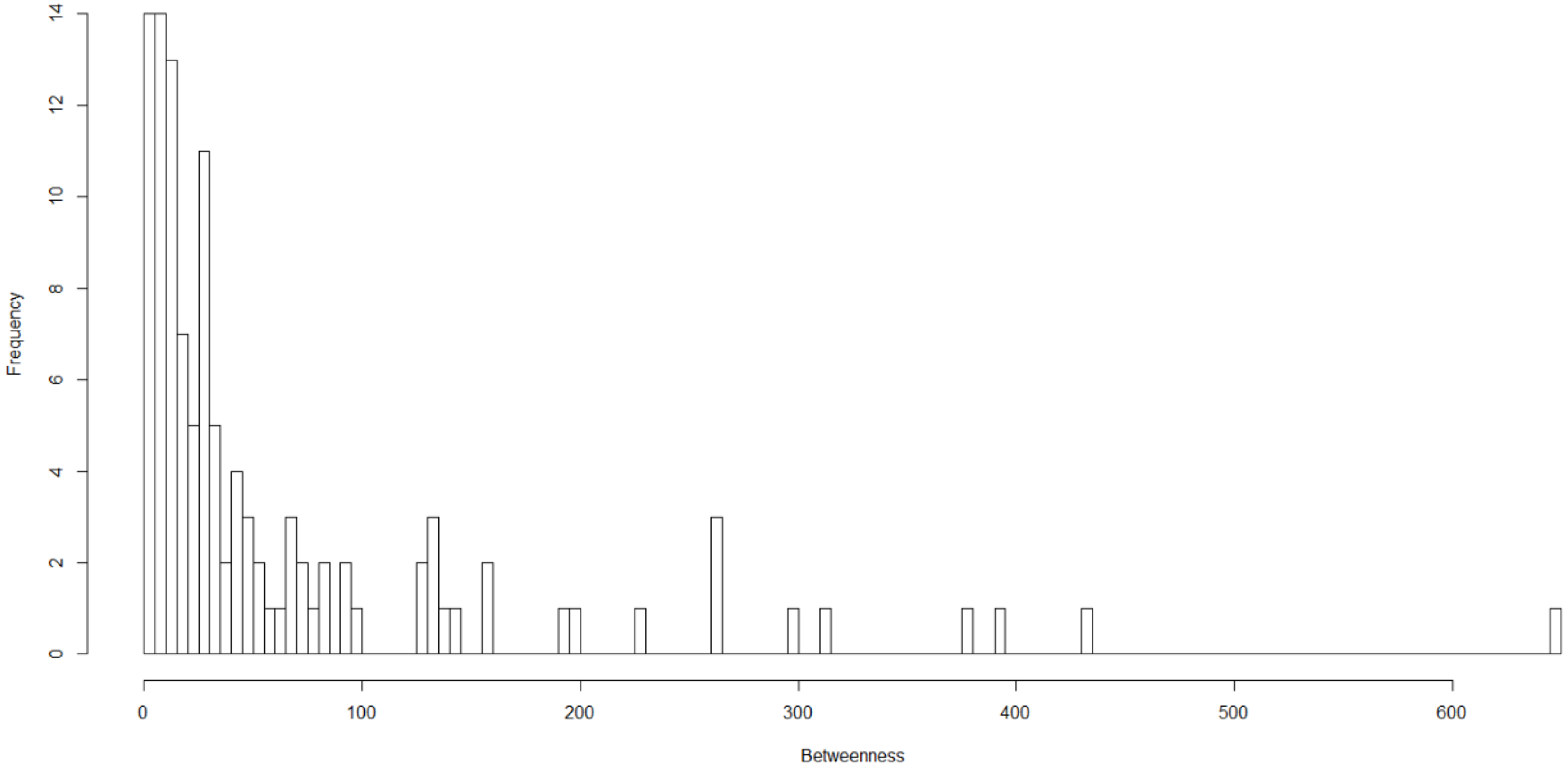
Supplementary Figure 5.1 Communities in the pig movement network through wholesale live pig markets in Guangdong in January and June 2016. Areas with a different color represent different communities in the network, and nodes with the same color belong to the same community.



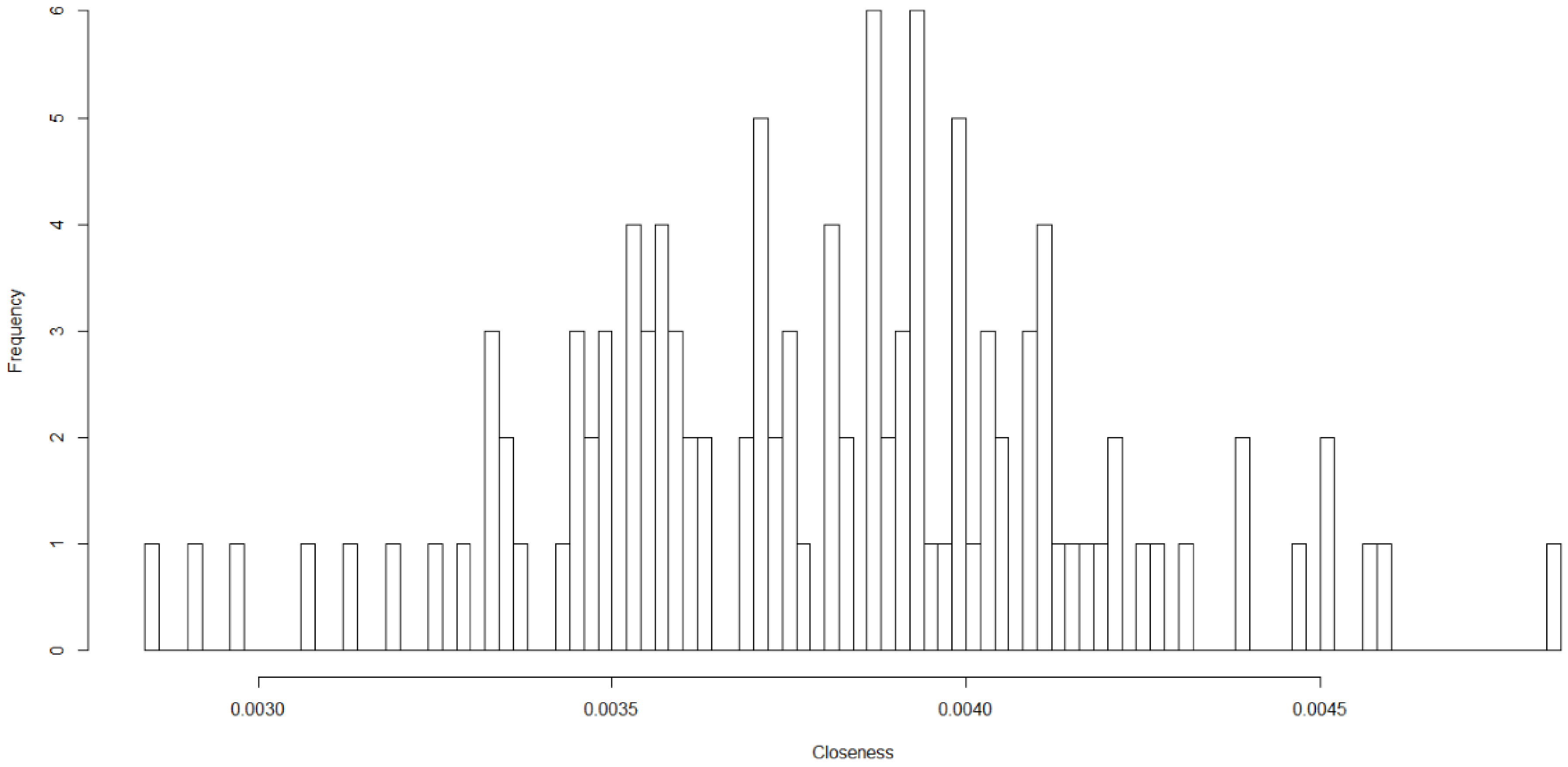
Supplementary Figure 5.2 The distribution of degrees of source counties in the combined static network of pig movement through wholesale live pig markets in Guangdong in January and June 2016.



Supplementary Figure 5.3 The distribution of betweenness of source counties in the combined static network of pig movement through wholesale live pig markets in Guangdong in January and June 2016.



Supplementary Figure 5.4 The distribution of closeness of source counties in the combined static network of pig movement through wholesale live pig markets in Guangdong in January and June 2016.



Supplementary Table 5.1 The degree, betweenness and closeness of each source county in the pig market trading network in Guangdong Province, 2016

County	degree	Betweenness	closeness
Sanshui	76	673.845	0.003745
Yuncheng	54	282.91	0.00346
Dinghu	85	919.031	0.003876
Jianghai	36	47.586	0.003257
Electric white	10	0	0.002967
Pengjiang	46	120.534	0.003367
Hepu	32	134.029	0.003215
New	43	89.974	0.003333
Four	80	773.088	0.003802
Kaiping	40	109.745	0.003289
Enping	54	169.576	0.00346
Huaiji	47	206.345	0.003378
Nanhai	34	44.231	0.003215
Lianzhou	12	2.748	0.002959
Yangchun	50	153.602	0.003413
Yangxi	26	23.637	0.003145
Yingde	44	144.877	0.003333
Longyuqu	10	0	0.002967
Cold water beach	8	0.462	0.002899
Pubei	7	3.138	0.002571
Gaoyao	24	22.674	0.003125
Fresh	44	81.639	0.003344
Guiping	10	0	0.002865
Xiangxiang	11	0	0.002907
Closed	26	38.288	0.003106
Huazhou	39	131.357	0.003289
Qingcheng	49	153.498	0.003401
Heshan	39	60.046	0.003279
Lu Chuan	36	178.41	0.003257
Huadu	25	13.504	0.003135
Xinxing	60	277.728	0.003534
Nansha	10	0	0.002882
Yunan	10	0	0.002882
Yangdong	36	37.917	0.003247
Taishan	31	17.68	0.003195
Xingan	15	3.777	0.002985
Camphor	25	30.527	0.003135
High security	10	0	0.002865
Conghua	4	0	0.00277
Fenyi	4	0	0.002786
Lechang	29	48.335	0.003185
Pingnan	6	3.75	0.002747
Taixing	9	0	0.00289
Maonan	3	0	0.002725
Fogang	18	5.232	0.003067
Changting	6	0	0.002801

County	degree	Betweenness	closeness
Hengxian	10	0	0.002967
Baiyun	57	378.2	0.003497
Linwu	12	2.265	0.002899
Qinbei	5	0	0.002825
Qiyang	6	0	0.002841
Jiangyong	8	0.462	0.002899
On the high	10	0	0.002865
Feng	4	0	0.002786
Foshanshixiaqu	5	0	0.002793
Qintang	5	0	0.00274
Nanxiong	29	48.354	0.003165
Ma Zhang	22	13.027	0.003086
Renhua	35	61.119	0.003236
Wengyuan	33	47.35	0.003205
Qujiang	30	36.742	0.003175
Luoding	10	4.479	0.002778
Gaoming	16	6.776	0.00277
Yangshan	34	54.171	0.003205
Zhongshan	10	0	0.002825
Lianjiang	34	78.909	0.003226
Suixi	22	18.111	0.003096
Yong'an	18	6.722	0.003067
Bobai	34	82.422	0.003236
Zhenjiang	20	8.872	0.002899
Gaozhou	21	8.177	0.002907
Yunan	9	2.712	0.002786
Shixing	33	53.825	0.003226
Xuwen	6	0	0.002591
Leizhou	10	0	0.00266
Wujiang	14	3.057	0.002801
Beiliu	16	17.137	0.003003
Lian Shan Zhuang Yao Autonomous	8	7.199	0.002907
Big	19	5.907	0.002793
Ruyuan Yao Autonomous	28	24.369	0.003175
Lotus	19	2.854	0.003077
Zhongshan	1	0	0.002381
Nankang	14	2.332	0.002717
Wuchuan	15	1.418	0.002841
Lingling	6	0	0.002639
Longnan	12	1.499	0.002674
Yangshuo	3	0	0.002545
Dingnan	15	8.674	0.002959
Leping	5	0	0.002817
Slope head	18	7.592	0.002899
Jiangcheng	13	0.806	0.002941
Rongxian	9	7.849	0.002874
Leiyang	22	27.371	0.003049
Changning	14	3.239	0.00295

County	degree	Betweenness	closeness
Dongguan	8	0	0.002865
Hengyang	32	64.884	0.003155
Steaming	9	0	0.00277
Hengdong	14	3.239	0.00295
Hengnan	14	1.739	0.002976
Lianping	9	0	0.002882
Jiedong	11	0.549	0.002924
Lianyuan	17	8.113	0.003003
Xinhua	17	9.945	0.002976
Ruijin	7	0	0.002809
Pingjiang	10	0	0.002907
Fengshun	3	0	0.002558
Jishui	7	0	0.002809
Lengshuijiang	4	0	0.002786
Double clear	9	0	0.00277
Xinfeng	10	0	0.00266
Yuanzhou	7	0	0.002809
Yunxi	5	0	0.00277
Anyuan	5	0	0.00277
Zixing	12	2.754	0.002924
Yushui	8	1.057	0.002833

CHAPTER 6: Infection and determinants of human and avian influenza in pigs in south China

Preface

In Chapters Three and Four of this thesis the contacts between pigs and pig industry workers in Guangdong Province were described, highlighting that local pig farmers and traders generally had a low awareness of the zoonotic risk of SI. These two groups were found to be taking inadequate steps to protect themselves from zoonotic SIVs in their work. These findings justify the monitoring of zoonotic strains of SIVs in the Chinese pig population. SI is one of the diseases targeted by national surveillance programs and this surveillance is designed to enhance the early detection of new zoonotic SIV strains. A targeted risk-based strategy for surveillance is required in order to maximise the benefit of limited resources and to enhance the early detection of emerging zoonotic SI strains. In Chapter Five, the SNA on the live pig movement offered clues for targeted surveillance and evaluated the impact of live pig markets on potential SI epidemics. However, little is known about the impact of other variables on SI, and it is not clear what factors are associated with the spill-over infection of human and avian influenza viruses to pigs. In this chapter, other variables, with a focus on meteorological, geographical and anthropogenic factors, were investigated to determine their potential roles in influenza infection in pigs in south China.

The manuscript outlined in this chapter is currently under review for publication in '***Preventive Veterinary Medicine***'.

Statement of Contribution

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Principal Author

Name of Principal Author (Candidate)	Yin Li
Contribution to the Paper	Conceptualised and developed the study, planned, collected and analysed the data, interpreted the results and wrote the paper.
Overall percentage (%)	35
Signature	Date: 15/06/2020

Co-Author Contributions

By signing the Statement of Contribution, each author certifies that:

vii. the candidate's stated contribution to the publication is accurate (as detailed above);
viii. permission is granted for the candidate to include the publication in the thesis.

Name of Co-Author	Emeritus Professor Ian Robertson
Contribution to the Paper	Supervised the study and provided critical comments to improve the interpretation of results, edited and revised the manuscript.
Overall percentage (%)	5
Signature	Date: 15/06/2020

Name of Co-Author	Dr Ding Fangyu
Contribution to the Paper	Planned, collected and analysed the data, interpreted the results and edited the manuscript.
Overall percentage (%)	35
Signature	Date:15/06/2020
Name of Co-Author	Emeritus Professor John Edwards
Contribution to the Paper	Provided critical comments to improve the interpretation of results, edited and revised the manuscript.
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Professor Huang Baoxu
Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	5
Signature	Date: 15/06/2020
Name of Co-Author	Professor Jiang Dong
Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Professor Zhang Guihong
Contribution to the Paper	Collected Surveillance data of SIV
Overall percentage (%)	5
Signature	Date:15/06/2020
Name of Co-Author	Dr Cai Chang
Contribution to the Paper	Provided critical comments to improve the manuscript.
Overall percentage (%)	2.5
Signature	Date:15/06/2020
Name of Co-Author	Ms Wang Qian
Contribution to the Paper	Collected and prepared data
Overall percentage (%)	2.5
Signature	Date:15/06/2020

Abstract

The coinfection of swine influenza (SI) strains and avian/human-source influenza strains in piggeries can contribute to the evolution of new influenza viruses with pandemic potential. This study analyzed surveillance data on SI in south China and explored the spatial predictor variables associated with different influenza infection scenarios in counties within the study area. Blood samples were collected from 7670 pigs from 534 pig farms from 2015 to 2017 and tested for evidence of infection with influenza strains from swine, human and avian sources. The herd prevalences for EA H1N1, H1N1pdm09, classic H1N1, HS-like H3N2, seasonal human H1N1 and avian influenza H9N2 were 88.5, 64.5, 60.3, 57.8, 12.9 and 10.3%, respectively. Anthropogenic factors including detection frequency, chicken density, duck density, pig density and human population density were found to be better predictor variables for three influenza infection scenarios (infection with human strains, infection with avian strains, and coinfection with H9N2 avian strain and at least one swine strain) than were meteorological and geographical factors. Predictive risk maps generated for the four provinces in south China highlighted that the areas with a higher risk of the three infection scenarios were predominantly clustered in the delta area of the Pearl River in Guangdong province and counties surrounding Poyang Lake in Jiangxi province. Identification of higher risk areas can inform targeted surveillance for influenza in humans and pigs, helping public health authorities in designing risk-based SI control strategies to address the pandemic influenza threat in south China.

6.1 Introduction

Disease arising from infection with influenza A viruses is one of the most widespread diseases of humans and animals (World Health Organization 2019). The influenza A viruses have a broad host spectrum, including humans, pigs, birds, tigers, dogs, domestic cats, horses, seals, whales and bats (Brown 2000, Yassine, Lee et al. 2013, Poole, Yu et al. 2014). Influenza virus is an RNA virus and hence genomic mutations occur frequently (Webster, Bean et al. 1992), with its segmented nature allowing the recombination of genes from different strains (Zhou, Senne et al. 1999, Kuntz-Simon and Madec 2009, Rajao, Walia et al. 2017). These mutations and recombinations result in the production of different subtypes and strains circulating in the field (Kuntz-Simon and Madec 2009). Furthermore immunity induced against one strain/subtype fails to confer cross-protection against different subtypes/lineages (Webster, Bean et al. 1992). These features of the virus make control of this globally distributed disease challenging.

Swine influenza (SI) is a highly contagious disease of pigs (Crisci, Mussa et al. 2013) with infected pigs displaying clinical signs of coughing, fever, and inappetence (Takemae, Tsunekuni et al. 2018). Although the clinical signs of SI in pigs are often mild, co-infection with porcine reproductive and respiratory syndrome (PRRS) and other diseases can result in high mortality (Nakharuthai, Boonsoongnern et al. 2008) and infection in pregnant sows can result in stillbirths (Wesley 2004). Besides the significant economic impact of SI to the pig industry, SI viruses (SIV) can also infect other species, including birds and humans (Hass, Matuszewski et al. 2011, McCune, Arriola et al. 2012, Zhu and Shu 2013, Bowman, Nelson et al. 2014), and spillover infection of SI strains to humans has become an emerging problem in public health (Gregory, Lim et al. 2001, Gray and Kayali 2009, Tang, Shetty et al. 2010, van der Meer, Orsel et al. 2010). Gene exchange between strains circulating in different species may lead to new epidemics in one or multiple species. Although each host species has several dominant influenza subtypes/strains circulating in its population, occasionally cross-species infection occurs (Yang, Qiao et al. 2012, Zhu and Shu 2013, Sikkema, Freidl et al. 2016). Pigs are susceptible to both avian and human influenza strains. In 2009, H1N1pdm09 was responsible for a pandemic, and subsequently this subtype has become established worldwide in the pig population (Keenliside 2013). The reassortment between SIVs and H1N1pdm09 has drawn attention as coinfection of pigs with SIV and avian/human-source influenza strains can contribute to the evolution of new influenza viruses with pandemic potential for humans (Zhu, Zhou et al. 2011, Hiromoto, Parchariyanon et al. 2012, Grontvedt, Er et al. 2013, Rajao, Walia et al. 2017, Chastagner, Bonin et al. 2019).

Surveillance of SI is not only crucial for animal health but also essential for preparing against a potential pandemic influenza threat, and is undertaken in many countries, including China (Vincent, Awada et al. 2014). South China is an area with vast populations of humans, birds and pigs. Previous studies in this area revealed complicated gene exchange between local SIVs and avian/human source viruses in pigs (Chen, Fu et al. 2014, Xie, Zhang et al. 2014, Yang, Chen et al. 2016, Ma, Wang et al. 2018). To detect newly emerging SIVs, especially strains with pandemic potential, pre-emptive and risk-based surveillance is needed. However, the frequency and distribution of SI infection, especially cross-species infection, in this area are still unknown. In this study, we take advantage of existing surveillance data to explore the frequency and spatial distribution of SIV and human and avian influenza virus strains in pig farms in south China. To inform future targeted SI surveillance, anthropogenic, meteorological and geographical factors associated with human and avian influenza viral infection in pigs were also explored. The findings of this study can help in the design of risk-based SI surveillance to address the pandemic influenza threat in south China.

6.2 Materials and Methods

6.2.1 Study design

The main objectives of this study were to describe the distribution of seropositive pigs to influenza strains in the sampled farms and to establish models to predict the influenza infection risk in counties in Guangdong, Guangxi, Jiangxi and Fujian provinces, south China. A pig with an antibody titer > 1:40 on the hemagglutination inhibition (HI) test was classified as seropositive, and a farm having at least one seropositive pig against a specific strain was defined as a case farm for that strain. For spatial modelling, a county was used as the study unit, with counties containing one or more case farms categorized as positive. The association of anthropogenic, meteorological and geographical factors with different scenarios, as listed in Table 6.1, of seropositivity to influenza A in piggeries and counties was investigated.

Table 6.1 Different influenza infection scenarios used in this study

Scenario	Definition	Code
An avian influenza infected farm	A farm containing one or more pigs that were seropositive to at least one avian influenza virus	aivInf
A multi-strain infected farm	A farm containing one or more pigs that were seropositive to at least two SIV strains	mltInf
A more-than-two-strain infected farm	Farms that had pigs that were seropositive to at least three SIV strains were defined as a more-than-two-strains infected farm	mltInf2
A more-than-three-strain infected farm	A farm containing one or more pigs that were seropositive to all four SIV strains	mltInf3
An AIV and human seasonal influenza virus coinfected farm	A farm containing one or more pigs that were seropositive both to at least one AIV subtype and human seasonal H1N1 influenza virus	aivhsInf
An H9N2 AIV and SIV coinfected farm	A farm having pigs that were seropositive to H9N2 AIV and at least one dominant SIV strains (including EA avian-like H1N1, H1N1pdm09 and classic H1N1)	h9sivInf
A county with AIV infection in local pig herds	A county with at least one pig herd infected with AIV strains (including H4N8, H6N6, H7N9, H9N2, H10N8 and H5N1)	AIV
A county with seasonal human flu infection in local pig herds	A county with at least one pig herd infected with seasonal human H1N1 influenza virus	SHH1N1IV
A county with pig herds positive to H9N2 AIV and SIVs	A county with at least one pig herd positive to H9N2 AIV and at least one SIV strain (including EA H1N1, H1N1pdm09 and classic H1N1)	H9SIV

6.2.2 Dataset collection

6.2.2.1 Blood Samples

Surveillance data on SI from 2015 to 2017 in south China were sourced from the Guangdong Key Laboratory for Zoonoses Prevention and Control, South China Agriculture University (SCAU). This laboratory has been offering diagnostic and consultant services to pig farms in south China since the 1990s and SI is one of the diseases that the laboratory focuses on. The results of testing of blood samples collected from 7670 pigs originating from 533 pig farms located in 71 counties of 12 provinces in south China from 2015 to 2017 were analyzed. No vaccines against SI had reportedly been used on these farms during the study period. The hemagglutination inhibition (HI) test was used to confirm influenza infection in pigs following an established protocol (Cao, Zhu et al. 2013). Information on the virus strains used in this study is summarized in Table 6.2. No cross-reactions in HI testing were observed between these strains.

Table 6.2 The virus strains used in the hemagglutination inhibition test in this study.

Strains	Subtype	Major host species
A/swine/Guangdong/SS1/2012	EA H1N1	Swine
A/Guangdong/1057/2010	H1N1pdm09	Swine/human
A/swine/Guangdong/L3/2009	Classic H1N1	Swine
A/Guangdong/NH1/2012	Seasonal Human H1N1	Human
A/swine/Guangdong/L22/2010	H3N2 HS-like	Swine/human
A/swine/Guangdong/K4/2010	H4N8	Avian
A/chicken/Guangdong/178/2004	H5N1	Avian
A/swine/Guangdong/K6/2010	H6N6	Avian
A/chicken/Guangdong/G2/2013	H7N9	Avian
A/chicken/Guangdong/V/2008	H9N2	Avian
A/chicken/Jiangxi/102/2013	H10N8	Avian

6.2.2.2 Spatial Predictor Variables

Several anthropogenic factors were chosen as predictor variables for different influenza infection scenarios in a county. The anthropogenic factors included in this study were: sampling frequency (number of farms sampled during the study period), hours of travel time by driving to a major city, chicken density, duck density, pig density, human population density, gross domestic product (GDP) and amount of night-time light (Table 6.3). Some of these factors have previously been linked to infection with several avian influenza viruses in poultry and humans (i.e., H7N9 and H5N1) (Gilbert, Golding et al. 2014, Li, Yang et al. 2015). Sampling frequency was added to the modelling to control for confounding. A detailed description of the anthropogenic factors included in this study can be found elsewhere (Ding, Fu et al. 2018, Gilbert, Nicolas et al. 2018).

Meteorological factors, including annual cumulative precipitation, maximum and minimum annual temperatures, and mean annual relative humidity (Table 6.3), were also included as predictor variables for the different influenza infection scenarios in counties. Based on the data set (V3.0) of daily climate values from Chinese surface stations, the ANUSPLIN-SPLINA software was employed to generate 1 km × 1 km gridded meteorological factors for the period 2015 to 2017.

A set of geographical predictor variables, including elevation (height above sea level), water cover area, distance (km) to water cover, distance (km) to a nature reserve and normalized difference vegetation index (NDVI), were also included in the modelling.

Water cover area, distance to water cover, distance to a nature reserve and NDVI were included as potential indicators to reflect the link between a given county and potential bird habitats. The first two datasets were derived from the 1 km × 1 km gridded land use in 2015 obtained from the website (https://http://www.resdc.cn/) of the Resource and Environment Data Cloud Platform, Institute of Geographical Sciences and Natural Resources Research (IGSNRR), Chinese Academy of Sciences. Based on the nature reserve boundary dataset downloaded from the Information Center of Ministry of Ecology and Environment of the People's Republic of China, the distance (km) between a given location and the nearest nature reserve was also calculated. The 2015 mean annual NDVI data with an 8 km × 8 km spatial resolution were obtained from the Global Inventory Modelling and Mapping Studies group (https://ecocast.arc.nasa.gov/). The data sources of all potential predictor variables modelled are summarized in Table 6.3.

Table 6.3 The variables included in the analyses.

Category	Predictor variable	Definition of the variable	Data source
Anthropogenic	Sampling frequency	Number of farms sampled in a county during the study period	Guangdong Key Laboratory of Zoonoses Prevention and Control in South China Agriculture University
	Travel time to a major city	Hours needed to travel to a city with a population of more than 50, 000.	European Commission Joint Research Center Global Environment Monitoring Unit (http://forobs.jrc.ec.europa.eu/)
	Chicken density	Total number of chickens per pixel (10 km^2)	Harvard Dataverse (https://library.harvard.edu/services-tools/harvard-dataverse)
	Duck density	Total number of ducks per pixel (10 km^2)	
	Pig density	Total number of pigs per pixel (10 km^2)	
	Human population density	The population density in a county (people per km^2)	National Bureau of Statistics of China
	Gross domestic product (GDP)	Total GDP of a county (Ten thousand yuan)	Global Change Research Data Publishing & Repository (http://www.geodoi.ac.cn/weben/)
	Night-time light	Range from 0 to 63, reflecting the development level of a county	The Earth Observation Group, NOAA (https://www.ngdc.noaa.gov/eog/)
Meteorological	Annual cumulative precipitation	Average annual cumulative precipitation (mm) for 2015 – 2017	China Meteorological Data Service Center (https://data.cma.cn/en)
	Maximum annual temperature	Average maximum annual temperature (°C) for 2015 – 2017	
	Minimum annual temperature	Average minimum annual temperature (°C) for 2015 – 2017	
	Mean annual relative humidity	Mean annual relative humidity for 2015 - 2017	

Category	Predictor variable	Definition of the variable	Data source
Geographical	Elevation	Averaged height above sea level (meters) of a county	The Consultative Group on International Agricultural Research Consortium for Spatial Information (http://www.un-spider.org/links-and-resources/institutions/consultative-group-international-agricultural-research-consortium-spatial-inform)
	Density of water cover area	The total area of all the natural and human-made water bodies divided by the total area of a county (per ha)	Resource and Environment Data Cloud Platform, Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences (http://english.igsnrr.cas.cn/)
	Distance to water cover	Distance (Km) to a natural or human-made water body	
	Distance to a nature reserve	Distance (Km) from the geometric centre of a county to the nearest nature reserve as defined by the government classifications	Information Center of Ministry of Ecology and Environment of the People's Republic of China (http://english.mee.gov.cn/)
	Normalized Difference Vegetation Index (NDVI)	The difference between the reflection value in the near-infrared band and the reflection value in the red-light band divided by the sum of the two. NDVI can reflect the background influence of plant canopy	Global Inventory Modeling and Mapping Studies group

6.2.3 Data analysis

6.2.3.1 Influenza infection in pigs in the sampled pig farms

The proportion of farms positive for each strain was calculated and the chi-square test used to compare the farm-level prevalence between strains. To demonstrate the transmission capacities of different influenza strains in pigs on a farm, the individual animal level seroprevalence for each strain in each seropositive farm were calculated, then for each strain, the mean and the 5% and 95% percentile of these individual animal level seroprevalences in seropositive farms were calculated to estimate the mean and 90% range of the individual animal seroprevalence of a strain in infected pig farms. An ANOVA was used to compare the individual pig-level seroprevalence for different SIVs or AIVs in the positive farms. The proportion of farms with multi-strain SIV coinfection (mltInf, mltInf2 and mltInf3), AIV infection (aivInf), AIV and human seasonal influenza coinfection (aivhsInf), and H9N2 and SIVs coinfection (h9sivInf) were also calculated. Basic R packages (R Core Team 2018) were used for these calculations. A case farm was a farm that contained one or more seropositive pigs to: AIVs; seasonal human H1N1; multiple strains of SIV at the same time; or H9 and SIVs at the same time. Counties that contained case farms were illustrated with maps developed with ArcGIS (Version 9.3, ESRI Inc., Redlands, CA, USA).

6.2.3.2 Spatial data preprocessing

In the present study, the WGS-84 geographical coordinate system was adopted (Slater and Malys 1998). In addition, all data, including county administrative boundary dataset obtained from the IGSNRR and the related predictor variables, were translated into a unified coordinate system. The related predictor variables were converted to county-scale datasets.

6.2.3.3 Modelling

The R (v 3.3.3) statistical programming environment was employed in the present study. The extension “gbm” and “dismo” packages were used to build boosted regression tree (BRT) models (Jiang, Wang et al. 2019). AIV, SHH1N1IV and H9SIV (see definitions in Table 6.1) were modelled and analyzed separately. For each infection scenario, the seronegative counties were randomly selected from the counties labelled with a 0, with twice as many negative counties selected compared to seropositive counties. To reduce the effect of negative county samples on the modelling process, the operation of randomly selecting counties with no farms containing seropositive pigs was conducted 50 times. Based on these operational steps an ensemble of 50 BRT models was fitted to increase the robustness of the analysis and to quantify the uncertainty of the modeling results. The values of the main parameters (i.e., tree complexity and learning rate) of all 50 BRT ensembles were included using the methods of Messina (Messina, Kraemer et al. 2016). In the present study, the area under the curve (AUC) was adopted as the accuracy evaluation index for the BRT models, and a 10-fold cross-validation method was employed to guard against over-fitting. In addition, the relative contribution (RC) indicator was calculated to quantify the contribution of each related covariate to the ensemble BRT models (Zheng, Jiang et al.). Based on the fitted BRT ensembles, the 95% confidence intervals (CI) of the RC for each related covariate were calculated.

Maps were developed using the established models to predict the relative risk of counties having the three influenza infection scenarios: AIV, SHH1N1IV and H9SIV. The risk levels were divided into ten categories from 0 (blue) to 1 (red) when developing maps. The marginal effect curves of sampling frequency in the ensemble BRT model fitted to AIV, SHH1N1IV and H9SIV were illustrated to determine which values to use when developing maps. The scope of the maps covered all the counties in Guangdong, Guangxi, Jiangxi and Fujian provinces.

6.3 Results

6.3.1 Influenza infection in local pig farms

The herd-level seroprevalence of different influenza strains is summarized in Table 6.4. Evidence of infection with all the tested strains, except for H10N10, was found on one or more sampled pig farms. In general, SIV strains (EA H1N1, H1N1pdm09, Classic H1N1 and H3N2 HS-like) had a higher herd-level seroprevalence than a human-source strain (Seasonal Human H1N1) and avian-sourced strains (H4N8, H6N6, H7N9, H9N2, H10N8 and H5N1). Among the SIV strains, EA H1N1 had the highest herd-level seroprevalence (88.5%), followed by H1N1pdm09 (64.5%), classic H1N1 (60.3%) and H3N2 HS-like (57.8%) ($P < 0.05$). One or more pigs were seropositive to seasonal human H1N1 in 12.9% of the sampled farms. For avian-source strains, H9N2 had the highest herd-level seroprevalence (10.3%), while the herd-level seroprevalences of the other strains were very low (< 3%) ($P < 0.05$).

Nearly one-third (32.6%) of the sampled farms contained pigs that were seropositive to the four SIV strains tested (Table 6.4), although individual pigs were not necessarily seropositive to the four strains. Antibodies to more than two SIV strains in pigs at a sampling were detected in 67.2% of the sampled farms. 9.2% of the farms contained pigs that were seropositive to H9N2 AIV and at least one SIV, while approximately 1% of the sampled farms contained pigs that were seropositive to seasonal human H1N1 influenza and at least one AIV strain (Table 6.4).

Table 6.4 Serological status of sampled pig farms in south China from 2015 to 2017

Influenza strain/Scenarios	Major host species affected by the influenza strain	Number of tested farms*	Number of farms that had seropositive pigs (%)	P value
H1N1pdm09	Swine/human	533	344 (64.5)	< 0.05
EA H1N1	Swine	416	368 (88.5)	
Classic H1N1	Swine	527	318 (60.3)	
H3N2 HS-like	Swine/human	533	308 (57.8)	
Seasonal Human H1N1	Human	295	38 (12.9)	-
H4N8	Avian	303	8 (2.6)	< 0.05
H6N6	Avian	191	1 (0.5)	
H7N9	Avian	311	3 (1.0)	
H9N2	Avian	532	55 (10.3)	
H10N8	Avian	83	0 (0)	
H5N1	Avian	210	1 (0.5)	
One or more pigs were seropositive to at least one avian influenza virus	-	533	63 (11.8)	-

Influenza strain/Scenarios	Major host species affected by the influenza strain	Number of tested farms*	Number of farms that had seropositive pigs (%)	P value
One or more pigs were seropositive to at least two SIV strains	-	533	358 (67.2)	-
One or more pigs were seropositive to at least three SIV strains	-	533	281 (52.7)	-
One or more pigs were seropositive to all the four SIV strains	-	533	174 (32.6)	-
One or more pigs were seropositive both to at least one AIV subtype and human seasonal H1N1 influenza virus	-	533	4 (0.8)	-
One or more pigs were seropositive to H9N2 AIV and at least one SIV strains (including EA avian-like H1N1, H1N1pdm09 and classic H1N1)	-	533	49 (9.2)	-

* Not all farms were tested for every strain

The individual animal level seroprevalence to each influenza strain on infected farms is summarized in Table 6.5. There was a significant difference in the animal level seroprevalence for the different SIV strains tested (F = 71.4, $P < 0.01$). EA H1N1 had the highest average animal level seroprevalence of 39.3%, followed by H3N2 (37.3%). H4N8 had a higher average individual animal prevalence (19.6%) than H9N2 (7.3%) in the infected farms ($P < 0.01$).

Table 6.5 Pig level seroprevalence to different influenza strains in infected pig farms sampled in south China from 2015 to 2017

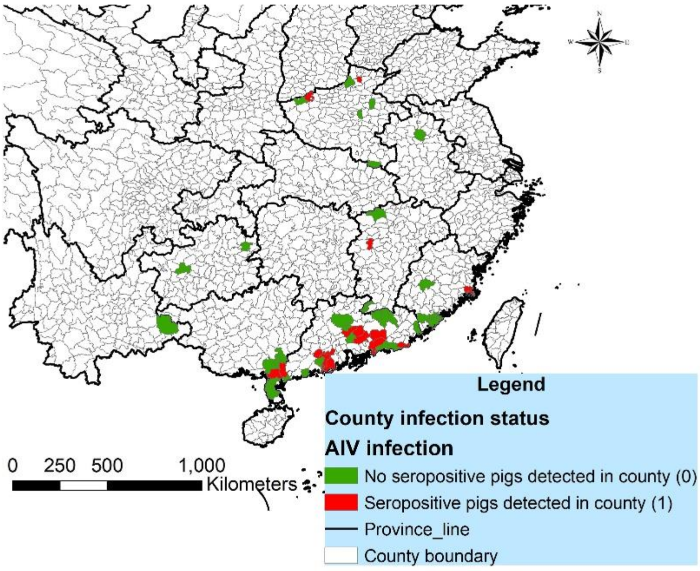
Influenza strain	Average animal level prevalence in infected herds (%)	5th and 95th percentile of individual prevalence in the positive farms
H1N1pdm09	21.6	6.3 – 50.0
EA H1N1	39.3	10 - 83.3
Classic H1N1	21.8	6.3 – 55.6
H3N2 HS-like	37.3	6.7 – 100.0
Seasonal Human H1N1	9.2	2.5 – 20.3
H9N2	7.3	1.9 – 15.0
H4N8	19.6	-*
H6N6	2.1	-*
H7N9	8.1	-*
H5N1	6.3#	-*
H10N8	-	-

*Not calculated as only 8 or fewer farms contained seropositive pigs

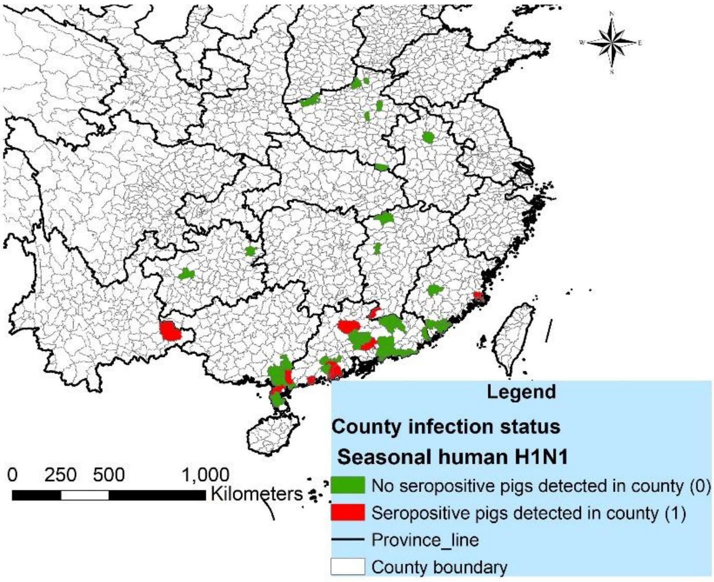
Only one farm contained a single positive pig of 16 sampled.

The counties which had pigs that were seropositive to different influenza strains and to multi-strain are displayed in Figure 6.1.

A


N
W
E
S
Legend
County infection status
AIV infection
🟩 No seropositive pigs detected in county (0)
🟥 Seropositive pigs detected in county (1)
Province_line
County boundary
0 250 500 1,000
Kilometers

B


N
W
E
S
Legend
County infection status
Seasonal human H1N1
🟩 No seropositive pigs detected in county (0)
🟥 Seropositive pigs detected in county (1)
Province_line
County boundary
0 250 500 1,000
Kilometers

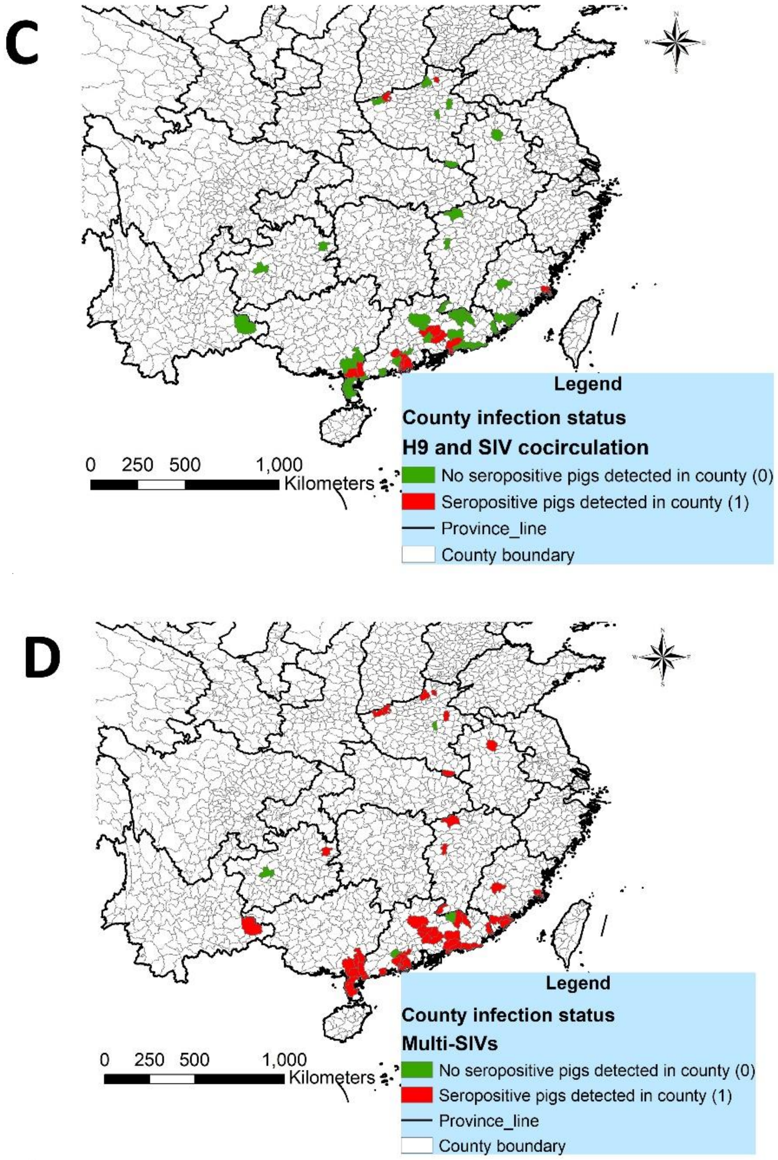


Figure 6.1 The counties that had pigs that were seropositive to different influenza strains and to multi-strain in Guangdong, Guangxi, Jiangxi and Fujian provinces.

6.3.2 The relative contribution of related covariates

The AUC values for the BRT model for AIV, SHH1N1IV and H9SIV were 0.72 (95% CI: 0.71-0.73), 0.85 (95% CI: 0.84-0.86) and 0.70 (95% CI: 0.68-0.71), respectively. The anthropogenic factors were the most important predictor variables in the ensemble BRT models, contributing 65.74% to the spatial variation for AIV, 83% to that for SHH1N1IV, and 67.04% to that for H9SIV (Table 6.6). Of the eight anthropogenic factors, the sampling frequency had the most notable RC, with 39.74% (95% CI 38.30-41.18) for the models fitted to the AIV dataset, 73.93% (95% CI 73.02-74.85) for the models fitted to the SHH1N1IV dataset and 44.57% (95% CI 43.33-45.70) for the models fitted to the H9SIV dataset. The RCs of each of the remaining seven anthropogenic factors were less than 10% in all infection scenarios. For the AIV-based fitted BRT models, chicken density (RC 8.45%, 95% CI 8.26-8.64), travel time to a major city (RC 5.56%, 95% CI 5.24-5.88), human population density (RC 3.37%, 95% CI 3.25-3.48) and duck density (RC 2.88%, 95% CI 2.79-2.97) were notable predictor variables. For SHH1N1IV-based fitted BRT models, human population density (RC 5.46%, 95% CI 5.21-5.71), and pig density (RC 1.18%, 95% CI 1.09-1.26) played a more important role than did chicken density (RC 0.87%, 95% CI 0.83-0.91), travel time to a major city (RC 0.69%, 95% CI 0.64-0.74), and duck density (RC 0.55%, 95% CI 0.52-0.57). In the H9SIV-based BRT ensemble, the notable anthropogenic predictor variables were, in decreasing order of RC values, travel time to a major city (RC 7.89%, 95% CI 7.54-8.22), duck density (RC 5.79%, 95% CI 5.66-5.91), chicken density (RC 3.50%, 95% CI 3.40-3.60), night-time light (RC 2.53%, 95% CI 2.47-2.50) and pig density (RC 1.50%, 95% CI 1.38-1.61).

Meteorological and geographical factors also contributed to the prediction of risk areas. The relative contributions of meteorological factors to predicting the risk of having AIV, SHH1N1IV and H9SIV in a county were 12.36, 6.82 and 20.62%, respectively. The meteorological factors contained four elements, of which annual cumulative precipitation contributed the most, both when predicting the risk of having AIV (4.77%; 95% CI: 4.49-5.05) and H9SIV (9.88%; 95% CI: 9.75-10.00) in a county. Geographical factors contributed 21.91% to the estimation of the AIV's risk in a county, 10.16% to that of SHH1N1IV and 12.32% to that of H9SIV. Among the five geographical factors, elevation was the most important variable for predicting the risk areas of AIV and SHH1N1IV, contributing 14.02% (95% CI 13.88-14.16) and 5.64% (95% CI 5.5-5.78), respectively.

Table 6.6 The relative contribution of related covariates predicting the risk of a county having pigs exposed to AIV, SHH1N1IV and H9SIV.

	Mean and 95% CI of the relative importance		
	AIV (%)	SHH1N1IV (%)	H9SIV (%)
Anthropogenic factors*	**65.74**	**83.00**	**67.04**
Detection frequency	39.74 (38.3-41.18)	73.93 (73.02-74.85)	44.57 (43.33-45.7)
Travel time to a major city	5.56 (5.24-5.88)	0.69 (0.64-0.74)	7.89 (7.54-8.22)
Chicken density	8.45 (8.26-8.64)	0.87 (0.83-0.91)	3.5 (3.4-3.6)
Duck density	2.88 (2.79-2.97)	0.55 (0.52-0.57)	5.79 (5.66-5.91)
Pig density	2.69 (2.59-2.79)	1.18 (1.09-1.26)	1.5 (1.38-1.61)
Human population density	3.37 (3.25-3.48)	5.46 (5.21-5.71)	0.43 (0.41-0.45)
Gross domestic product (GDP)	1.06 (0.98-1.13)	0.1 (0.09-0.11)	0.83 (0.78-0.87)
Night-time light	1.99 (1.9-2.09)	0.22 (0.21-0.24)	2.53 (2.47-2.5)
Meteorological factors*	**12.36**	**6.82**	**20.62**
Annual cumulative precipitation	4.77 (4.49-5.05)	1.83 (1.76-1.9)	9.88 (9.75-10)
Maximum annual temperature	4.35 (4.14-4.56)	2.67 (2.55-2.8)	2.24 (2.1-2.37)
Minimum annual temperature	1.19 (1.09-1.29)	1.42 (1.35-1.49)	6.23 (5.87-6.58)
Mean annual relative humidity	2.05 (1.99-2.11)	0.9 (0.84-0.97)	2.27 (2.19-2.34)
Geographical factors*	**21.91**	**10.16**	**12.32**
Elevation	14.02 (13.88-14.16)	5.64 (5.5-5.78)	1.16 (1.11-1.21)
Water cover area	4.24 (4.08-4.39)	1.55 (1.48-1.63)	2.64 (2.54-2.74)
Distance to water cover	0.86 (0.79-0.92)	1.25 (1.18-1.32)	2.61 (2.44-2.78)
Distance to nature reserve	1.21 (1.16-1.25)	1.22 (1.14-1.31)	3.32 (3.22-3.4)
Normalized Difference Vegetation Index (NDVI)	1.58 (1.5-1.65)	0.5 (0.48-0.53)	2.59 (2.52-2.65)

*Sum of relative contribution for each category.

The marginal effect curves of sampling frequency in the ensemble BRT model fitted to AIV, SHH1N1IV and H9SIV are displayed in Supplementary Figure 6.1. Sampling frequency had a strong positive association with the presence of AIV as the sampling frequency increased to 9 after which no further effect on the response was observed. A similar trend was also found in the modeling and analysis for H9SIV. When the sampling frequency initially increased, the probability of SHH1N1IV-positive did not alter. However, the probability of a county having pigs that were seropositive to AIV increased rapidly when the sampling frequency increased from 3 to 5. Overall the relationships between sampling frequency and the presence of these three infection scenarios were similar.

6.3.3 Estimating relative risk level for the survey zones

By setting the sampling frequency to 5 and 9, the final predicted relative risk level maps of AIV, SHH1N1IV and H9SIV in the counties in Guangxi, Guangdong, Fujian and Jiangxi provinces were obtained (Figure 6.2). The sampling frequency was set at 5 and 9 because the marginal analysis indicated that the association between the sampling frequency and the presence of interested scenarios did not increase after reaching these sampling frequencies (see supplementary Figure 6.1). Using a sampling frequency of 9 (Figures 6.2 B, D, F), the predicted risk levels for these three scenarios in the four provinces were higher than that with a sampling frequency of 5 (Figures 6.2 A, C, E), since the risk gradually increased from blue to red when the risk levels were divided into ten categories from 0 (blue) to 1 (red). By adopting a threshold value of 0.5 to distinguish high-risk from low-risk areas, only the central part of Guangdong was classified as a high risk area for AIV at a sampling frequency of 5 (Figure 6.2 A), while at a sampling frequency of 9 it extended to most parts of Guangdong, the northern counties of Jiangxi (cities of Nanchang, Ji'an, Xinyu and Shangrao), central and coastal areas of Fujian (cities of Fuzhou, Quanzhou and Nanping) and the southern part of Guangxi (Nanning and Yulin cities) (Figure 6.2 B). For SHH1N1IV, the high risk areas were more widely distributed with higher risks in the coastal areas of Guangxi and Guangdong provinces and the northern region of Jiangxi province at a sampling frequency of 5 (Figure 6.2 C), and at a sampling frequency of 9, the high risk area expanded to include most parts of Guangdong and Jiangxi provinces, the western area of Guangxi province and the coastal and western regions of Fujian province (Figure 6.2 D). For H9SIV, the high-risk areas were significantly smaller than for the two previously mentioned viruses. Xinxing County in Yunfu City of Guangdong province was the only area at high risk under a sampling frequency of 5 (Figure 6.2 E), while at a sampling frequency of 9 the area of Guangdong province under high risk was still relatively broad (Guangzhou, Qingyuan and Jiangmen cities) and the other three provinces also had some high risk regions, but overall the level of risk was not as high as for the other viruses examined (Figure 6.2 F).

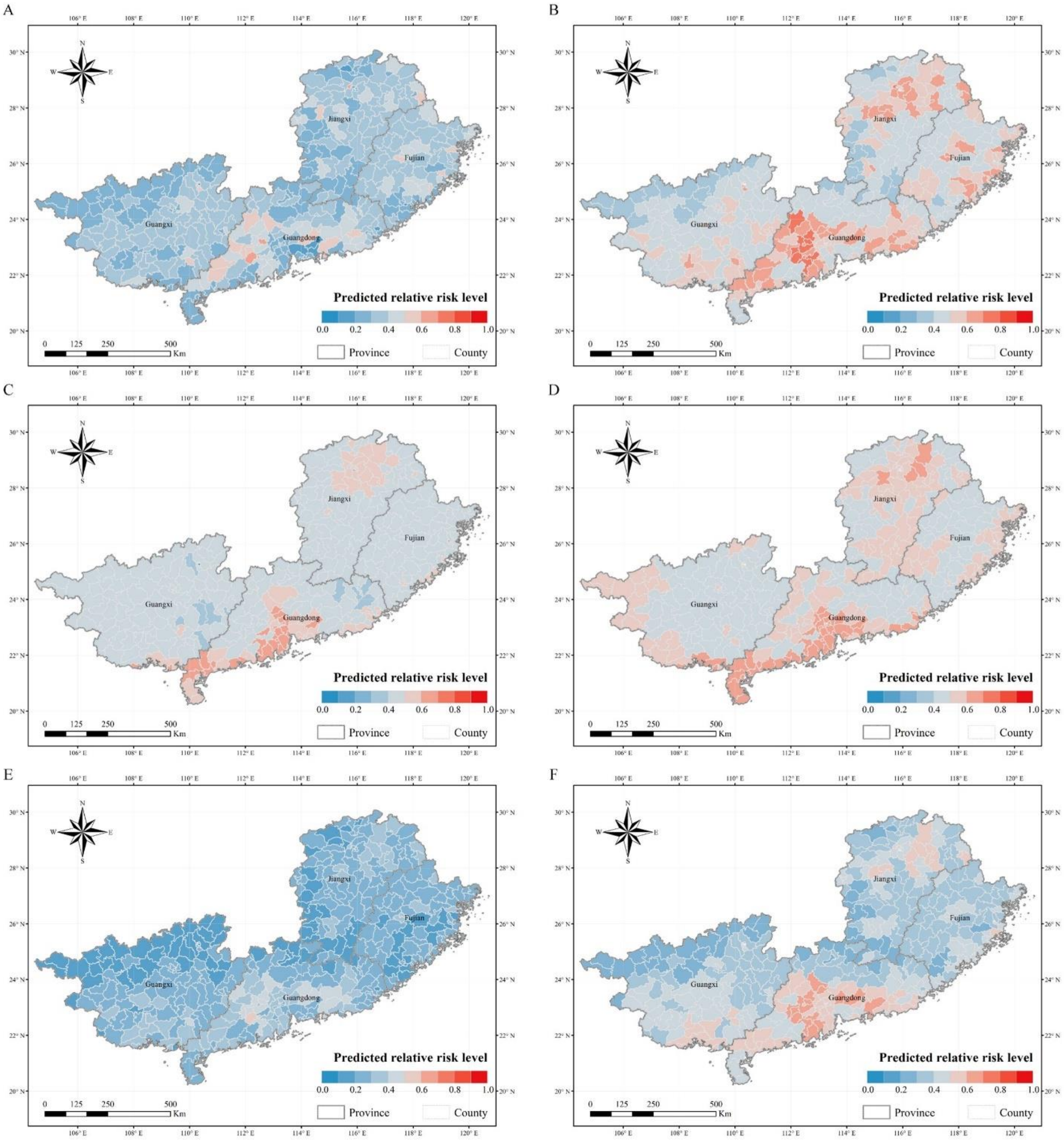


Figure 6.2 The estimated relative risk level of AIV, SHH1N1IV and H9SIV in Guangdong, Guangxi, Jiangxi and Fujian provinces, China.

6.4 Discussion

This study found a complicated pattern of influenza virus infection in pigs in south China with a high herd level prevalence and widespread distribution of SI within the region. Some pigs and herds were found to be co-infected with SIV and avian/human source influenza strains which would explain why gene reassortment among SIV strains has been commonly observed in this area (Qiao, Liu et al. 2014, Xie, Zhang et al. 2014, Yang, Chen et al. 2016). The animal level seroprevalences in infected herds varied between the virus strains examined, potentially reflecting their different transmission abilities. It is possible that when an avian/human influenza strain infects pigs, the virus either gradually establishes in the pig population, or dies out due to an inability to adapt to the different species. Monitoring the prevalence of avian/human strains in pigs would be useful to predict which strains are establishing in pigs.

Based on passive surveillance data and the BRT modeling framework, the complex links among three infection scenarios and related covariates were analyzed and quantified. For each infection scenario, the fitted ensemble BRT models were combined with different sampling frequencies to estimate the relative risk level for infection in different counties in this area, enhancing the health authorities' capacity to target critical areas for surveillance and to develop focused control strategies. Several studies have explored the spatial patterns of the monitoring targets, especially for AIV (Moura, Perdigao et al. 2009, Nelson, Philippe et al. 2011, Trock, Burke et al. 2015, Delabouglise, Choisy et al. 2017). For instance, the spatial characteristics of H5N1 and H7N9 human influenza infection cases were analyzed in research which explored the effects of meteorological factors on the infections (Li, Rao et al. 2015). Another study included several environmental variables (human population density and presence of water bodies) to explore the spatial epidemiological characteristics of H7N9 and H5N1 human infections (Li, Yang et al. 2015). Chicken density, duck density and travel time to a major city were also included in research conducted by Gilbert, Golding et al. (2014), revealing the reasons for the geographic distribution of H7N9. Compared with previous studies, the current research used more spatial predictor variables (17) in order to investigate the current situation in south China. In addition, sampling frequency was added as a covariate and was found to have a significant impact on the prediction results, which had been overlooked in the data collection and modeling processes performed by other researchers.

There are limitations in using passive SIV surveillance data, including having samples only from some of the counties in the provinces investigated and incomplete information about the sampled farms. Furthermore, the regional herd prevalence may actually be lower than the value estimated from this study due to potential overrepresentation of farms which have been sampled to confirm the diagnosis of animals displaying clinical signs typical of SI. However even with these limitations, passive surveillance of SI is still adopted in many countries as it is more economical and practical than active surveillance (Delabouglise, Antoine-Moussiaux et al. 2016). Some studies have found that data from large-scale passive surveillance can provide useful information on the distribution of diseases (Amezcua, Pearl et al. 2013, Simon, Larsen et al. 2014, Strutzberg-Minder, Tschentscher et al. 2018). In the future, data from well-designed active surveillance on SI should be collected and used to further examine the predictive factors identified from the current study.

Machine learning (ML) has been used in disease prediction in recent years (Bhatt, Gething et al. 2013). ML could allow the exploration of a broader scope of variables, and may be better for predicting an event than with traditional models (Boeckel, Thanapongtharm et al. 2012). However, it is often challenging to explain the logical relations between the dependent and independent variables (Elith, Leathwick et al. 2008). With a high demand for disease prediction in the field and with more algorithms being developed, ML will likely play a more important role in identifying "hot spots" for disease surveillance. We suggest big data should be used to explore more potential ways to predict the risk distribution of SI, as well as other diseases of animals and humans. A relevant database with good quality, current and timely updated data is also required for this to be possible.

The results arising from the current study would also benefit the implementation of active surveillance on SI. In China, surveillance on SI has been included in the annual animal disease surveillance plan (Ministry of Agriculture and Rural Affairs of China 2019). One of its priorities is to detect influenza strains that are capable of infecting humans. Active surveillance in pigs, involving collecting blood samples to evaluate the seroprevalence of different strains and collecting nasal swabs to isolate viruses that are circulating in the field, is currently being undertaken in south China. However, there are limitations in the current active surveillance activities undertaken in China. Firstly, convenience sampling has been used when selecting areas to sample; Secondly, samples are mostly collected at slaughterhouses which could lead to a delay in detecting new emerging strains in the field and predominantly limits sampling to apparently healthy animals of market age; and Thirdly, there is low sensitivity in virus isolation from samples collected from finisher pigs as this age group is less susceptible to SI compared with piglets or weaners (Takemae, Parchariyanon et al. 2011, Ozawa, Matsuu et al. 2015). These limitations in the current SI surveillance activities would lower the chance of detecting influenza strains with zoonotic potential. To improve the sensitivity for detecting such strains, farms in areas with a higher risk of spill-over influenza infection should be targeted for sampling in the future.

In this study, the frequency of SIV strains and human and avian influenza infection in pig farms in south China were described. Several anthropogenic, meteorological and geographical factors associated with human and avian influenza virus infection in pigs were identified. Counties in the delta area of the Pearl River in Guangdong province and counties surrounding Poyang Lake in Jiangxi province were identified as potential target areas for active surveillance of SI to detect zoonotic SIVs. The findings of this study can benefit risk-based SI surveillance in south China to reduce the impact of SI in pigs and for the prompt detection of recombinant influenza viruses with pandemic zoonotic potential.

6.5 Acknowledgements

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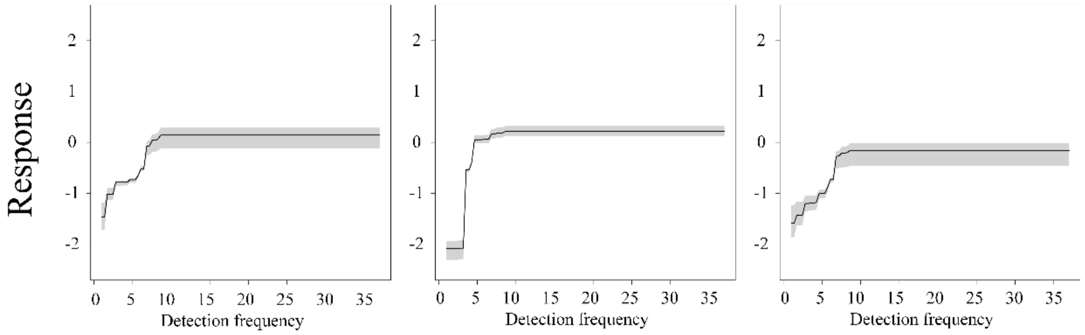
6.6 Supplementary Material

Supplementary Figure 6.1 The marginal effect curves of sampling frequency in the ensemble BRT model fitted to AIV (left), SHH1N1IV (middle) and H9SIV (right).



CHAPTER 7: General discussion

Internationally swine influenza has a high economic impact on the pig industry and has been rated as one of the most important pig diseases globally (Hernandez-Jover, Taylor et al. 2012, Er, Lium et al. 2014, Er, Skjerve et al. 2016). Swine influenza virus also poses a significant threat to public health, with zoonotic transmission to humans reported in many countries, including China (Yang, Qiao et al. 2012, Wang, Qi et al. 2013, Zhu and Shu 2013, Bowman, Nelson et al. 2014, Sikkema, Freidl et al. 2016). In China the large pig and human populations, a high prevalence of SI in pig farms, the presence of many new emerging variants of SIV and frequent contacts between humans and pigs, make the local control of SI in both pigs and people challenging.

In recent years several emerging zoonotic diseases, including HPAI H5N1, H7N9, SARS and COVID-19, have thrived in China, especially in south China (Zhong, Zheng et al. 2003, Yu, Shu et al. 2006, Ke, Mok et al. 2017, Mitchell 2020). Some of these diseases, such as COVID-19, became pandemics resulting in significant impact to human health, society and the economy at a national and international level (Mitchell 2020). Understanding the epidemiology of these highly contagious diseases is key to their effective control. The findings of the research reported in this thesis will not only benefit the control of SI in pigs but also assist in reducing the risk of zoonotic transmission of SIV to humans in south China.

A systematic analysis of SI in south China was conducted in the research reported in this thesis to support evidence-based control of the disease within the study area. Although this study primarily focused on Guangdong Province in south China, the findings are likely to be applicable to other regions/provinces of China. In this study the husbandry and biosecurity practices adopted by local pig farmers were described, the prevalence of farmer-perceived SI at the farm level evaluated and the risk factors for farmer-perceived SI analysed. The contacts between pigs and local pig industry workers, including pig farmers, pig traders and trade workers, and the industry workers' knowledge and beliefs about SI were also investigated. The movement network of live pigs through the wholesale live pig markets in the study area was analysed to identify the source counties with the highest risk of having SI, if there was an epidemic of SI spreading via the market trading system. Different strategies were evaluated to direct risk-based disease control and spatial modelling was employed to explore determinants for scenarios in counties that could lead to the future development of a zoonotic influenza strain in pigs. In this final chapter, the findings of this study are reviewed, areas that need further research are highlighted and limitations of the current study discussed.

7.1. The husbandry and biosecurity practices in pig farms in Guangdong Province

Very few studies have explored the husbandry and biosecurity practices adopted in Chinese piggeries and this deficiency was addressed in Chapter Three, where the husbandry and biosecurity practices adopted by commercial piggeries in Guangdong Province were described. These findings can help understand the current and potential transmission pathways of SI between local piggeries.

The study found that there were frequent direct and indirect contacts of pigs, people, vehicles and fomites from the surveyed farms with those of other premises. For example, 57% of the surveyed farms introduced pigs in the year preceding the survey, with an average introduction frequency of twice a year (Chapter 3). The introduced pigs may spread SI and potentially other diseases between local farms when the infected imported pigs were mixed with the pigs on farm. Swine influenza could also be introduced into a farm at the time of selling pigs since proper control of the movement of buyers was lacking on many farms. Different "types" of piggeries were found to have different selling rates with farrow-to-weaning farms selling pigs, on average, 200 times a year compared with an average of 6 times for fattening farms. To the knowledge of the author, no other study has presented statistics on the introduction and selling practices of Chinese pig farms. These values could be used for quantitative risk analysis on the spread of pig diseases in local pig farms.

Biosecurity in pig farms has been very topical in China in recent years, especially since the incursion of ASF (Wang, Sun et al. 2018, Zhou, Li et al. 2018); however on-farm biosecurity in Chinese pig farms has rarely been reviewed. Several significant limitations in the biosecurity implemented in pig farms in Guangdong Province were highlighted in this research (Chapters 3 and 4). Firstly, many farms failed to quarantine introduced pigs properly with 35% of the surveyed farms not implementing quarantine on all of the pigs introduced to the piggery. Secondly, the movement of people not associated with the day-to-day running of the enterprises onto the farms was not restricted. This is evident in that 30% of the surveyed farms allowed buyers to select and then load their purchased pigs, and 72% of these farms didn’t require the people loading these pigs to change their boots before they entered the piggery. It is likely that the proportion of farms which allow buyers to enter the piggeries may be higher than 30%, because 12 of the 15 surveyed trade workers reported that they entered piggeries to collect pigs themselves. Swine influenza virus can be transmitted between farms on contaminated boots or clothes of visitors (Torremorell, Allerson et al. 2012, Allerson, Cardona et al. 2013). A study conducted in six commercial Chinese pig farms reported that 11.6% of swabs of surfaces that were likely to be touched by humans, including piggery gates and walls, and 4.8% of faecal or slurry samples were positive for influenza A by qRT-PCR (Anderson, Ma et al. 2018). Influenza A virus can retain its infectivity for 14 days in slurry at 20 ℃ (Botner and Belsham 2012). This duration is sufficient to allow the spread of SI via contaminated clothes/boots since the surveyed traders visited farms, on average, once every three days. These results suggest that more restrictions should be implemented to prevent the indirect spread of SI and other contagious diseases via the movement of people. To be more specific, buyers should not be allowed to enter the piggeries, or they should be required to undertake biosecurity measures, such as changing or cleaning and disinfecting their clothes and boots, before and after entering piggeries. Taken together, these results indicate that biosecurity gaps on pig farms in Guangdong Province may have facilitated the spread of SI between and within local farms.

7.2. Prevalence and risk factors of farmer reported swine influenza infection

As mentioned in the literature review, prior to the study reported in this thesis, there was limited information on the farm-level prevalence of SI in China, and most of the previous reports were based on biased sampling, including samples collected from slaughterhouses. For the first time a study was undertaken to determine the farm-level prevalence of SI in south China (Chapter 3). Nearly 60% of the surveyed farmers believed SIV infection had been present in their pigs in the six-month period prior to the survey. A similar high farm-level prevalence has been reported in other countries, including the USA, Norway, Spain and UK (Mastin, Alarcon et al. 2011, Simon-Grife, Martin-Valls et al. 2011, Corzo, Culhane et al. 2013, Er, Skjerve et al. 2016).

It was surprising that no prior study had analysed risk factors for SIV infection in Chinese pig farms, even though the molecular epidemiology of SIVs has been intensively studied in China, including in the same location as the current study (Bi, Fu et al. 2010, Liu, Wei et al. 2011, Qiao, Liu et al. 2014, Zhou, Cao et al. 2014, Yang, Chen et al. 2016). Several risk factors were identified as being associated with SI in the surveyed Chinese pig farms. One of the interesting findings was that the presence of poultry was associated with farmer perceived SIV infection (OR 3.24, 95% CI: 1.52–6.94). A similar result was also reported by Simon-Grife, Martin-Valls et al. (2011). This result may be explained by the fact that AIV from poultry can occasionally infect pigs. Several studies have reported infection of pigs from south China with AIV (Ninomiya, Takada et al. 2002, Song, Xiao et al. 2010). Another important finding from this research was that the entry of wild birds into a piggery increased the likelihood of SI being reported by farmers. This finding supports evidence from a previous observation that wild waterfowl can transmit influenza A virus to pigs (Karasin, Brown et al. 2000). Influenza viruses may be spread to pigs via direct contacts between wild birds and pigs, or indirectly via faecally contaminated feed or water (Karasin, Brown et al. 2000, Torremorell, Allerson et al. 2012, Anderson, Ma et al. 2018). The current study found that 89% of the surveyed farms had a pond on the farm. Even though only a small proportion (1%) of farms actually used these ponds as sources of drinking water for pigs, 18% of the farms did use pond water to flush effluent in the piggeries. As ponds are likely to attract wild birds, and AIV can survive in pond water for days or even months (Webster, Yakhno et al. 1978, Ito, Okazaki et al. 1995), there is the potential for aerosolisation and dispersal of virus during flushing. An evidence-based regulation for SI control should be established to discourage or ban raising poultry on commercial pig farms and to construct piggeries to prevent access by wild birds. This could include not allowing free-roaming poultry on pig farms by applying official assessments/certification, such as the “Standardized demonstration farm for livestock and poultry breeding” (Ministry of Agriculture and

Rural Affairs 2018), and requiring adequate screening/barriers to prevent the entry of wild birds.

In this study, the lack of biosecurity for workers before they entered piggeries was identified as a risk factor for SIV infection on farms. Similarly, Wang, Wen et al. (2016) reported that the entry of visitors was a risk factor for porcine reproductive and respiratory syndrome in Chinese pig farms. These results highlight the need for improving biosecurity procedures for visitors to and workers on pig farms. All people should undertake necessary biosecurity procedures before entering the piggeries, such as ideally showering or at the very least hand-washing, changing their “street” clothes and boots to farm provided attire, walking through a disinfectant fogging room and wearing face masks (Alarcon, Monterubbianesi et al. 2019). The risk factors for farmers’ perceived SIV infection identified in this research should be considered when developing a control strategy for SI. However, different control measures will vary in their cost and may not always be practical or achievable on individual farms. When implementing control and preventive practices, it is important to consider the cost-benefit of each of the planned control measures, however the benefit of implementing strengthened biosecurity practices is that it will also reduce the likely entry of many other infectious and potentially costly diseases to the pig industry.

7.3. The risk of zoonotic transmission of swine influenza at the human-pig interface

Pig industry workers have a higher risk of contracting SI than people without occupational exposure to pigs (Myers, Olsen et al. 2006, Yin, Rao et al. 2014, Ma, Anderson et al. 2015). However, the epidemiology of SI at the human-pig interface in China has rarely been investigated, and in particular the knowledge of pig traders, their beliefs about SI and their hygiene practices were unknown prior to the study reported in Chapter Four.

In the current study the role of local pig industry workers in zoonotic SIV infection in south China was investigated. The practices adopted by local traders which may facilitate the spread of SI between farms should be considered when designing control programs against the disease. For example, 80% of the interviewed trade workers said they would enter a piggery when collecting pigs. This is a risky practice with the potential for introducing SI onto a farm, and other studies have similarly highlighted that visitors can spread a range of diseases between farms (Brennan, Kemp et al. 2008, Grontvedt, Er et al. 2013, Lichoti, Davies et al. 2017). To reduce the chance of introducing SI to pig farms, new ways of selecting pigs for sale/purchase should be developed on the local pig farms. Both buyers and producers could take advantage of social applications (APPs), such as WeChat, QQ and Skype, to conduct real-time video communication/inspection to select pigs. WeChat offers the advantage in China that it is already used by more than half a billion Chinese (Zeng, Deng et al. 2016) and would not require additional investment by users, although a smart phone is a prerequisite to use this and other video communication software.

In this study it was found that some traders (25% of those surveyed) would return pigs displaying clinical signs characteristic of SI to the source herd. Regulations need to be developed and implemented in local live pig markets and slaughterhouses to prevent this practice. These clinically affected pigs could be slaughtered and processed immediately, as pork meat from influenza infected pigs is believed to be free from SIV (Vincent, Lager et al. 2009). However, the offal, including the lungs, blood and brain, of these animals should be condemned/rendered and not used for human or animal consumption as SIV has previously been detected in these organs of infected pigs (Janke 2014). However, the disadvantage of processing these pigs for human consumption, as opposed to total condemnation and rendering, would be an increased risk of acquiring SIV by the workers in the slaughterhouses. To address this risk, these pigs should be slaughtered separately and the workers slaughtering and processing these pigs required to wear proper personal protection equipment, such as wearing N95 face masks, and be required to work at a safe speed to minimise the potential for infection. An alternative could be that these pigs are transported directly to rendering facilities in slaughterhouses, although this would require compensation to be provided for its successful implementation and acceptance by the industry.

In this research, only 33.7% of the interviewees thought that SIV could infect humans. This highlights the limited knowledge of the interviewees about SI. Another study on the knowledge of pandemic (H1N1) in 2009 also reported limited knowledge about the disease by the general public in China, even though the virus had caused a pandemic when the study was conducted (Lin, Huang et al. 2011). Poor hygienic practices were adopted by many of the local pig industry workers, including 82.6% of respondents advising that they would continue to work if they had mild flu symptoms, and less than 40% of the interviewees would always wear gloves/masks when contacting pigs. These findings confirm that the risk of zoonotic SIV infection is high in local pig industry workers, particularly given the high frequency of SI in local pig farms (Chapters Three and Six). Consequently there is a need for systematic surveillance of SI in both pigs and people in south China and adoption of improved hygienic practices, such as wearing face masks and gloves, by all pig industry workers when contacting pigs.

Workers who continue working when displaying mild flu symptoms were more likely to not be aware of the zoonotic risk of SI (OR = 3.80, 95%CI: 1.38 – 11.46) and interviewees who had a lack of awareness of the zoonotic risk of SI were more likely not to use PPE when contacting pigs (OR = 3.19, 95%CI: 1.67 - 6.21), highlighting the poor knowledge/awareness about the disease by many local pig industry workers. Similarly Lin, Huang et al. (2011) reported an association between a poor level of knowledge about pandemic (H1N1) in 2009 and the adoption of risky practices. Key gaps in the knowledge of pig industry workers about SI were identified in this study, and these gaps should be targeted in focused educational initiatives in the future. To promote good knowledge about SI to local pig industry workers, simple leaflets with key information, such as SIVs can infect humans and wearing a face mask and gloves can provide protection when contacting pigs, should be developed and delivered to local pig farms, slaughterhouses and live pig markets. Local public and animal health authorities should be responsible for delivering extension material and training to improve the knowledge and awareness of local pig farmers about the disease. A study on malaria in the Democratic Republic of Congo showed that education was the most important factor that led to using bed nets by local villagers to reduce exposure to the mosquito vector (Ndjinga and Minakawa 2010). Similarly Bailey, Gamble et al. (2018) reported that a short lesson on rabies improved the knowledge and attitudes of school children in Malawi on the disease for at least 9 weeks after delivery. It has also previously been observed that a health education program for Thai farmers resulted in a significant improvement in the adoption of safe working practices by the farmers (Rattanaselanon, Lormphongs et al. 2018). An appropriately designed educational program on the impact of SI and the role biosecurity and improved hygiene can play in its prevention, would likely be a very cost-effective means of minimising the disease’s impact to the general community and the pig industry.

7.4. Pig movement in the live pig markets in south China

Other studies have described the trade and poor hygienic practices adopted in live bird markets in south China (Martin, Zhou et al. 2011, He, Liu et al. 2014); however prior to the study reported in this thesis no similar study had been conducted on the live pig markets in the country. In Chapter Five, the trade volume and frequency, number of traders and supply counties and catchment areas of the wholesale live pig markets in Guangdong Province were explored. The findings of Chapters Four and Five indicated that the live pig markets in Guangdong Province are potential hubs for SI, as well as other diseases of pigs, facilitating spread between pig farms in south China. At least 151 counties in Guangdong, Hunan, Guangxi, Jiangxi, Fujian and Henan provinces were connected to these markets and more than 14,000 batches of pigs were traded during the two-month study period in 2016. In addition, the trade practices adopted by local traders could facilitate the spread of contagious diseases between pig groups from different farms. For example, the traders often mixed pigs from different farms to make a trade, and they would spend 15 hours on average to sell a batch of pigs. A particularly risky practice adopted was that unsold pigs were kept for several days by the traders enhancing the potential transmission of pathogens between pigs sourced from different farms. Even though these markets may have played a significant role in the spread of epidemics, it is important to bear in mind that closure of local markets may not be practical or ideal. Traditionally Chinese have preferred eating fresh pork and this is a key driver for the need for live pig markets. Unless there is a change to accepting chilled meat by the community it will be difficult to remove live animal markets from China (Lin, Zhang et al. 2017). The closure of markets may potentially alter the movements of live pigs, possibly resulting in unexpected consequences. This occurred when live bird markets were closed in China with the aim to control H7N9. New movement patterns of birds occurred resulting in the spread of H7N9 to rural areas adjacent to the cities (Li, Wang et al. 2018). A better alternative to closure would be to implement risk-based control according to the movement network of pigs and people associated with these markets. The areas with high connectivity should be identified because they would be expected to have a higher risk of an epidemic through this market trade network. The results presented in Chapter Five indicated that the supply counties with highest connectivity in the live pig market trading network were located in the north, centre and southwest of Guangdong Province. This finding highlights that pigs and piggeries in these areas should be specifically targeted for sampling, as opposed to random sampling, to enhance the early detection of new emerging SIVs. In this study, different strategies were also compared to illustrate the benefit of using risk-based intervention in containing an epidemic spreading via the market trading network. The results of this research highlighted that isolating the nodes with the highest betweenness and degree scores would be a key feature of reducing the magnitude of a potential epidemic. This recommendation is in line with other SNA studies that have been conducted on the movement of sheep in the UK (Kiss, Green et al. 2006) and the movements of sheep, cattle and deer between farms in New Zealand (Marquetoux, Stevenson et al. 2016). The models established in the current research justify that a risk-based strategy for control should be undertaken to quickly contain an epidemic. For example, at the early stage of an epidemic, such as COVID-19 or emerging human influenza, targeted sampling and movement restrictions based on SNA findings should be employed. This was highlighted in a recent study on COVID-19 in China where the number of cases in provinces was strongly associated with the number of emigrations from Wuhan to these provinces (Chen, Zhang et al. 2020). In conclusion, these findings offer valuable insights for decision-makers in an emergency response to an epidemic, particularly as they are often faced with the challenges of insufficient diagnostic capacity/capability and limited financial resources.

7.5. Spatial predictor variables associated with SI in counties in south China

Spatial predictor variables have been analysed in other species for a range of infectious diseases, including anthrax, rabies, peste des petits ruminants (PPR), and bluetongue (Mayo, Gardner et al. 2012, Tenzin, Dhand et al. 2012, Kracalik, Malania et al. 2013, Gao, Liu et al. 2019). In terms of spatial predictor variables for influenza A, studies have identified anthropogenic, meteorological and geographical factors associated with infection in poultry and humans (Ward, Maftei et al. 2008, Paul, Tavornpanich et al. 2010, Chen and Chen 2014, Kim and Pak 2019). However, the spatial variables associated with influenza infection in pigs had not been studied prior to the research reported in this thesis and this study was also the first attempt to link anthropogenic, meteorological and geographical factors and human/avian sourced influenza infection in pigs at a spatial scale in counties in south China (Chapter Six).

The current study found that elevation above sea-level was the most significant variable for predicting the presence of infected herds/seropositive pigs to AIV strains (relative importance: 14.02, 95%CI: 13.88-14.16) and also for infected herds/seropositive pigs to human seasonal influenza H1N1 (relative importance: 5.64, 95%CI: 5.5-5.78). A possible explanation for this might be that elevation (up to 200 m) is associated with increased densities and/or interactions of pigs, humans and birds within the study area. Although no similar studies have explored the association between elevation and SI in pig farms, a study in Europe reported that conversely a lower elevation was highly correlated with HPAI H5N1 in wild birds. In that study the authors hypothesised that lower flatter areas had more water sources which attracted waterfowl, resulting in mixing and spread of virus in this population (Si, Wang et al. 2010). The current study found that the density of chickens (relative importance: 8.45, 95%CI: 8.26-8.64) was the second most important predictor variable for AIV in pigs, and human population density the second most important predictor variable for human H1N1 in pigs (relative importance: 5.46, 95%CI: 5.21-5.71). Similarly, several other studies have reported that poultry and human density were both associated with outbreaks of H5N1 and H7N9 (Gilbert, Chaitaweesub et al. 2006, Chen and Chen 2014, Artois, Jiang et al. 2018).

The population density of chickens was more important than the density of humans, ducks and pigs in predicting the risk of having pigs seropositive against AIV strains on a farm. This supports the likelihood that chickens on pig farms are, not surprisingly, the most important sources of AIV infection for pigs. This was supported by the findings reported in Chapter Three where the presence of poultry on a farm was a risk factor for farmer' perceived SI.

7.6. Limitations and recommendations

There were several biases and limitations to the research reported in this thesis. Firstly, in Chapter Three, the diagnosis of SI and categorisation of a piggery as infected (positive) was based on the perceptions/observations of the farmers. The reliability of the case definition is heavily dependent upon the farmers' knowledge about SI because the diagnosis was rarely confirmed by a veterinarian or by laboratory testing. Secondly, the SNA undertaken as part of this research only included the movement of pigs via the live pig trade markets. The majority (approximately 80%) of live pigs move from farms to slaughterhouses in Guangdong Province (P. Chen, personal communication, July 10, 2018) and these movements were not analysed in this study. In addition, the movements of live pigs, including weaners and piglets between farms for grow-out purposes, were also not investigated. Thirdly, data from a laboratory offering diagnostic services to piggeries were used for the spatial modelling (passive surveillance data), as currently there are no available active surveillance data on SI from south China.

Because of these biases and limitations, it is recommended that in future:

- Additional studies using the results of laboratory/diagnostic tests to categorise SI infected and non-infected herds are undertaken to confirm the associations between the risk factors for farmer-perceived SI and laboratory confirmed infection with SI on local farms.

- Educational programs should be developed to enhance the knowledge about SI by the people involved in the pig industry in south China. Practical hygienic practices that would reduce the risk of transmission of influenza A between pigs and between humans and pigs and vice versa should be introduced to local pig industry workers. In particular the risk of zoonotic infection should be emphasised to traders and trade workers associated with the live pig markets. Methods to minimise the risk factors for SI in pigs and people, as identified in this study, should be developed and included in educational materials for local farmers and traders. In conjunction with these recommendations hygiene regulations should be developed and implemented to minimise transmission of SIV at live pig markets.
- To understand other potential pathways for the spread of SI between pig farms in south China, SNA should also be conducted to explore the movement networks of live pigs within the area. These movement data should include pigs moving from farms directly to slaughterhouses and pigs moving between farms.
- A comprehensive qualitative or quantitative risk analysis targeting the risk of zoonotic SIV infection in local pig industry workers should be conducted based on the findings of the current research. Through the use of sensitivity

analysis the trading patterns that need targeted interventions in local live pig markets system could then be identified.

- Additional studies on the spatial predictors of avian/human influenza infection in pigs using active surveillance data of influenza A viruses in pigs are needed to confirm the associations between the predicting factors for avian/human influenza infection in pigs. New variables, such as the densities of local wild waterfowl and migrating birds, could be explored in these new models.
- The findings of the research reported in this thesis highlight the need for improved surveillance for influenza A in both pigs and pig industry workers in south China. Commercial pig farms located in counties with high connectivity, as determined by the movement network of live pigs that are traded via local markets, and in counties of the delta area of the Pearl River in Guangdong Province and those surrounding Poyang Lake in Jiangxi Province should be targeted for SI surveillance.

7.7. Conclusions

This thesis has generated important information that: described the husbandry and biosecurity practices adopted on pig farms in Guangdong Province; demonstrated the presence of biosecurity gaps on pig farms and in live pig markets that potentially would lead to the spread of SI in Guangdong Province; identified a SI herd prevalence of 60% in six months of 2015 based on farmers’ perceptions about the disease; identified three risk factors (wild birds being able to enter piggeries, presence of poultry on the farm, and lacking disinfection procedures for workers before they enter piggeries) associated with SIV infection in pig farms in Guangdong Province; analysed the risk of zoonotic SIV infection at the interface between local pig industry workers and their pigs; analysed the movement pattern of pigs through the live pig markets network in Guangdong Province; and explored the anthropogenic, meteorological and geographical risk factors for human/avian influenza infection in pigs in south China. The findings presented in this thesis help further our understanding of the epidemiology of SI in south China and will improve the ability to control SI in pigs and humans in China.

Appendices

Appendix 1

Questionnaire for pig farmers

Objectives

- To record the management, biosecurity and trading behaviours in the pig farms involved in this project
- To collect information on relevant risk factors for SIV infection on pig farms

Farm-（　）Sampling Authority:_________ Recorder:____Tel:

Farm Address: village___town __ county ___ prefecture ___province		
Name of the farm		Tel：
Production information	1. Start time for breeding: Year of 2. Farm type: ☐ Fattening ☐ Self-reproducing ☐ Breeding ☐ Other: 3. Breed: ☐ Yorkshire ☐ Landrace ☐ 3-hybrid ☐ Duroc ☐ Other: 4. Pigs inventory _____head, including sows:____head, gilts:____head, Piglets:____head, fattening: ______head, and boars: ____ head 5. The raising pattern for fattening pigs: ☐all-in-all-out on farm; ☐all-in-all-out on single shed ☐mix batches in one piggery ☐other 6. Did you introduce any pigs during the last 12 months:☐Yes ☐No（Turn to Q9） How many times were pigs introduced: _____How many head were introduced: Source of introduction: ☐from breeding farms directly ☐ from middle man ☐from live pig market ☐other source: __________________ 7. Did you implement quarantine when pigs were introduced: ☐Yes ☐No ☐Sometimes 8. Did you perform any specific practices during period of quarantine for introduced pigs??: ☐observe only ☐observe and test ☐ it depends 9. In the last 12 months how many times did you sell pigs:_____How many head did you sell?: 10. Do you sell all of the pigs from a pen at one time? ☐Yes ☐No	

	☐Sometimes 11. When you sell pigs, who is responsible for picking up the pigs and loading them onto trucks: ☐our employees ☐buyers ☐ both 12. Do buyers change clothes before entering the producing area?: ☐Yes ☐No ☐Sometimes 13. Do buyers change their boots before entering the producing area?: ☐Yes ☐No ☐Sometimes 14. Do your employees shower and/or disinfect their footwear after loading and before handling pigs remaining in the herd?: ☐Yes ☐No ☐Sometimes 15. Have you seen pigs from other farms on the collection trucks before your pigs are loaded?: ☐Yes ☐No ☐not sure 16. What kind of environment is the farm in: ☐ Village ☐ Rural Area ☐ Other (please specify):
Management	17. Do you record abnormal situations such as reduced production, sick or dead pigs? ☐ No ☐ Yes ☐ Sometimes 18. Do you hire any veterinarians? ☐ No ☐ Full-time vet ☐ Part-time vet 19. Do the employees in producing area shift among different sheds ☐ Yes ☐ No 20. How often do you disinfect the pig houses? At least: ☐ Once a week ☐ Once a month ☐ Once every 3 months ☐ Never 21. What chemical do you usually use for disinfection?: 22. Where is your pig-feed sourced?: ☐ Purchase it myself ☐ The vendor sends it to the piggery ☐ Purchase the components and mix on the farm☐ all of the above 23. How often did you usually buy feed for your pigs? once every days
Biosecurity	24. What is surrounding your pig-farm? ☐ village ☐ cropland ☐ mountains ☐ other: 25. Are the living area(s) for workers and the pig production area(s) separated? ☐ No ☐ Yes 26. Is there a disinfection pool at the entrance of pig farm? ☐ No ☐ Yes

	27. Are visitors/vehicles allowed to enter the producing areas? ☐ No (go to Q30) ☐ Yes ☐ Sometimes 28. Are visitors/vehicles disinfected before they enter the piggery? ☐ No ☐ Yes ☐ Sometimes 29. Are visitors or vehicles allowed to enter production areas? ☐ No ☐ Yes, by changing boots ☐ Yes, by changing clothes ☐ other: 30. Other species on the farm? ☐ No (turn to Q37) ☐ dog (turn to Q31-33) ☐chicken ☐duck ☐ geese ☐ other: 31. Can the dog(s) contact pigs directly? ☐ No ☐ Yes ☐ Sometimes 32. Can the dog(s) contact the pigs' feed or drinking water? ☐ No ☐ Yes ☐ Sometimes 33. Do you feed the dog(s) raw poultry meat or pork? ☐ No ☐ Yes ☐ Sometimes 34. What type of poultry do you have on the farm? ☐ egg poultry ☐ meat poultry☐ both 35. Why are the poultry kept? ☐ self-consumption ☐ sell ☐ both 36. From where do you source poultry? ☐ LBMs ☐villages nearby ☐breeding farm ☐ Self-reproducing 37. Is there any water pools/ponds/dams on the farm? ☐ Yes ☐ No(turn to Q) 38. Do you take water from these sources pool to drink pigs? ☐ Yes ☐ No 39. Do you take water from these pools/ponds/dams to flush piggeries? ☐ Yes ☐ No 40. Have you ever seen any wild birds on the farm? ☐ No ☐ Yes 41. Can the wild birds enter into the piggery buildings? ☐ Yes ☐ No ☐ not sure 42. Do you have any facilities/practices to protect against wild birds entering the piggery? ☐ No ☐ Yes 43. Do you have disposal facilities for dead pigs? ☐ No ☐ Yes
Human-pig interface	44. Do the employees eat poultry meat? ☐ Yes ☐ No ☐ sometimes 45. Where do they get this poultry meat from? ☐ retail LBMs nearby ☐ wholesale LBMs ☐ poultry of their own 46. Do you feed pigs swill? ☐ Yes ☐ No 47. Have any of your staff had the flu (influenza) in the last 6 months

☐ No ☐ Yes

48. If so, what did they do?
☐ Clinic/hospital ☐ Took medicine ☐ Took a rest ☐Other

49. Did any workers still work on the farm if they were only mildly sick?:
☐ No ☐ Yes ☐ Sometimes

50. If so, did they wear a face-mask while working in the piggery? ☐ No ☐ Yes ☐ Sometimes

51. In the last 6 months have you seen any of your pigs sick with signs of difficult breathing, coughing, or discharge from the mouth or eyes? ☐ No(turn to Q55) ☐ Yes

52. If so, how many pigs in total were in the group :____, and how many of these were sick:
When did they get sick:________, and how long did the sickness last? days:

53. Was it diagnosed as SIV infection? ☐ No ☐ Yes

54. Who did the diagnosis?
☐ vets on farm ☐ official vets ☐ service company ☐Other:

55. Have you ever heard of Swine Flu? ☐ No ☐ Yes ☐ Don't know

56. Do you think it is an important pig disease? ☐ No ☐ Yes ☐ Don't know

57. Do you think it can cause pigs to die? ☐ No ☐ Yes ☐ Don't know

58. How do you treat with the pigs with flu?
☐ Sale ☐ Medicine treat ☐ No treat

59. When treating sick pigs, do workers wear face-masks and gloves? ☐ No ☐ Yes

60. Do you think swine flu can cause people to get the flu?
☐ No ☐ Yes ☐ Don't know

61. Are workers vaccinated against influenza every year? ☐ No ☐ Yes

Sampling information

62. Number of samples: ____in total, including_____swabs, _____serum samples

63. Storage condition:

64. Coding of the samples:

65. Among them: The coding _________ are sows;________are gilts
_______ are piglets; _______are fattening pig;________ are boars

Note: This questionnaire is used for an epidemiological survey only, your information will not be released to any other person or third party.

Appendix 2

Questionnaire for pig traders

No. of this interview：   Institute:

Interviewer: phone number:

Name of the interviewee：_______ phone number:

Name of the interview place

Address:__province __county __ township __village

Altitude _____ longitude

Trade practices	1. Working experience: have been trading pigs since the year of 2. What kind of pigs do you trade: ☐finishers ☐ weaners ☐both 3. What is (are) the size of the farm(s) from where you buy pigs: ☐ < 100 pigs ☐ 100-500 pigs ☐ > 500 pigs ☐ include all these categories 4. How many farms have you visited to buy finishers in the preceding 30 days?___ ； How many farms have you visited to buy weaners in the preceding 30 days? 5. How many batches of pigs are transported each month?__; How many pigs are in a batch? (head) __; How many farms are needed to make up a saleable batch? What is done with leftover pigs? Where do you purchase pigs from?：☐Contact pig farms themselves ☐contracted farms ☐middlemen ☐other: 6. Where do you sell pigs to? ☐Sell live pigs to slaughterhouses ☐Slaughtered by slaughterhouses and then sell meat themselves ☐Sell live pigs to meat sellers ☐Sell live pigs to other live pig traders ☐Sell live pigs to farms ☐other: 7. Trucks for transport: ☐Self-owned ☐Rented ☐other: 8. How many workers do you hire?__; What is the daily salary of a worker?__RMB/day 9. Do you have another occupation? ☐No ☐veterinary consultant ☐feed seller ☐livestock medicine seller ☐other:
Human-pig interface	10. Approximately how often would you visit at a pig farm? Once every__days 11. Do you wear gloves/masks when loading pigs? ☐No ☐Always ☐Sometimes 12. Do your workers wear gloves/masks when loading pigs? ☐No ☐Always ☐Sometimes 13. Would you enter a piggery to collect pigs? ☐No ☐Always ☐Sometimes 14. Do you require any certificate when transporting pigs? ☐No ☐Health certificate ☐Don't know 15. Did you have flu in the preceding 3 months? ☐No ☐Yes ☐Not sure 16. What would you do if you got the "flu"? ☐Go to see a doctor ☐Take some pills ☐Just have a rest without any medical

treatment
☐others:

17. Would you continue to work if you had a mild case of the flu? ☐No ☐Yes
18. Do you know a disease called swine influenza (SI)? ☐No ☐Yes
19. Do you think SI is a significant disease in pigs? ☐No ☐Yes ☐Don’t know
20. Do you think SI can kill pigs? ☐No ☐Yes ☐Don’t know
21. Do you think SI can infect humans? ☐No ☐Yes ☐Don’t know
22. Are you vaccinated against seasonal flu each year? ☐No ☐Yes
23. In your opinion how would your peers deal with stressed pigs that were reluctant to walk?
    ☐Sell as normal ☐Sell at a lower price ☐Sell after treatment using antibiotics ☐Return to the original farm ☐other:

Note: This questionnaire is used for an epidemiological survey only, your information won’t be released to the third party.

Contact address: No.369 Nanjing Road, Qingdao, Shandong, China Animal Health and Epidemiology Center

Contact Person: Yin Li Telephone: 85648638

Appendix 3

Questionnaire for pig trade workers

No. of this interview：________ Institute:

Interviewer: phone number:

Name of the interviewee：_____age:___ phone number:

Name of the interview place

Address:_province __county _ township __village

Altitude _____ longitude

Trade practices	1. Working experience: have been doing this job since the year of 2. How many days do you work in a month?___ ； How many hours do you work each day? 3. On how many days would you visit at least one farm? Once every___days 4. Usually, how many batches of pigs do you transport each day?__; How many pigs in total do you transport each day? _heads; 5. Which month is the busiest month?
Human-pig interface	6. Do you raise pigs or poultry at home? ☐No ☐Yes 7. Do your co-workers raise pigs or poultry at home? ☐No ☐Yes ☐Not sure 8. Do you wear gloves/masks when loading pigs? ☐No ☐Always ☐Sometimes 9. Would you enter piggery to collect pigs? ☐No ☐Always ☐Sometimes 10. Do farmers ask you to change your boots before entering their piggery? ☐No ☐Always ☐Sometimes 11. Do farmers ask you to change your clothes before entering their piggery? ☐No ☐Always ☐Sometimes 12. Do farmers ask you to undergo any disinfection procedure? ☐No ☐Yes If yes, what is the disinfection procedure? 13. Did you have flu in the preceding 3 months? ☐No ☐Yes ☐Not sure 14. What would you do if you got the “flu”? ☐Go to see a doctor ☐Take some pills ☐Just have a rest without any medical treatment ☐other: 15. Would you continue to work if you had a mild case of the flu? ☐No ☐Yes 16. Do you know a disease called swine influenza (SI)? ☐No ☐Yes 17. Do you think SI is a significant disease in pigs? ☐No ☐Yes ☐Don’t know 18. Do you think SI can kill pigs? ☐No ☐Yes ☐Don’t know 19. Do you think SI can infect humans? ☐No ☐Yes ☐Don’t know 20. Are you vaccinated against seasonal flu each year? ☐No ☐Yes

	21. In your opinion how would your peers deal with stressed pigs that were reluctant to walk? ☐Sell as normal ☐Sell at a lower price ☐Sell after treatment using antibiotics ☐Return to the original farm ☐other:

Note: This questionnaire is used for an epidemiological survey only, your information won’t be released to the third party.

Contact address: No.369 Nanjing Road, Qingdao, Shandong, China Animal Health and Epidemiology Center

Contact Person: Yin Li Telephone: 85648638

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