Determining the Incoming Prevalence of Type A Influenza in Exhibition Swine and Characterizing the On-Farm Swine Management Practices Associated with Type A Influenza.

THESIS

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Abstract

Swine play a key role in the evolution and ecology of influenza A virus (IAV) infecting humans as pigs are a host species in which reassortment of the IAV segmented genome commonly occurs. Due to the distinctive management practices under which they are reared and the way they are displayed for show, exhibition swine provide a critical human-swine interface allowing for the bidirectional zoonotic transmission of IAV. During agricultural fairs, these exhibition swine come into contact with not only their handlers/owners but also large numbers of other swine and the general public. Previous IAV surveillance in these unique settings occurred at the end of the fairs, after IAV had the opportunity to spread through the exhibition swine population. Little was known about the prevalence of IAV among pigs when they first arrive at exhibitions. These swine that are shedding IAV serve as the pathogen source, leading to infections in other pigs and people during the course of the fairs. To estimate the geographical location of exhibition swine in the Midwestern United States, the number of exhibition swine per county was compiled during 2013 and a heatmap was generated for the six participating states, showing a concentration of exhibition swine in Indiana and Ohio. In 2014, snout wipes were used to sample pigs during the first day of nine agricultural exhibitions in Indiana and Ohio. Samples were screened for the matrix protein gene of IAV using real-time reverse transcription polymerase chain reaction (rRT-PCR). Positive samples were
inoculated onto Madin-Darby canine kidney cells for virus isolation. The sampling
detected an IAV prevalence of 1.5% (52/3,547) among swine arriving at exhibitions. In
addition, a survey was administered to the families of exhibitors to determine the on-farm
management history of the exhibition swine. From the nine exhibitions, a total of 480
surveys were collected and correlated to 614 swine. Results of the survey revealed that
during a single year exhibition swine frequently move between multiple exhibitions,
which creates a pathway for widespread pathogen dissemination. From the prevalence
sampling, movement of swine through chutes during entry to fairs was identified as a
possible transmission point for pathogens between entering swine. Snout wipe results and
surveys were linked to assess if there was an association between IAV detection in
arriving swine and the surveyed on-farm management practices with which those swine
were raised. Participants that hosted and open house or sale had 3.933 times the odds of
having an IAV positive pig compared to the odds of participants that did not host an open
house or sale. Overall, this research yields a better understanding of the epidemiology of
IAV in exhibition swine and allows for improved prevention of IAV transmission
between swine and humans at agricultural fairs. This study illustrates that a small number
of pigs arrive at the fair shedding IAV, identifies a possible transmission point for IAV,
and expands upon the limited knowledge that is known about exhibition swine
management.
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CHAPTER 1: Literature Review

1.1 Influenza A virus background
Influenza A viruses (IAV) are enveloped viruses in the *Orthomyxoviridea* family with a single-stranded, negative-sense RNA genome that is comprised of eight independent gene segments (Lamb, 2007). The 11 proteins that these segments encode are neuraminidase (NA), hemagglutinin (HA), matrix protein 1 (M1), matrix protein 2 (M2), polymerase base 1 (PB1), polymerase base 2 (PB2), polymerase acid (PA), nucleoprotein (NP), nonstructural protein 1 (NS1), nuclear export protein/nonstructural protein (NEP/NS2) and polymerase basic 1-F2 (PB1-F2). Surface proteins HA and NA, depicted in Figure 1, are used for the subtyping of IAV, and both glycoproteins play important roles in the virus life cycle. Currently there are 18 HA subtypes (H1-H18a) and 10 NA subtypes (N1-N10) identified in host populations.

*Figure 1.1: A simplified structure of influenza A virus (Horimoto & Kawaoka, 2005)*
(Fouchier et al., 2005, Tong et al., 2013). HA is responsible for virus receptor-binding to sialylated host cell receptors, mediates membrane fusion, and is the target for infectivity-neutralizing antibodies (Skehel, 2000). Its counterpart surface protein NA, cleaves sialyl residues allowing newly enveloped IAV to leave the host cell membrane (Moscona, 2005).

The M1 protein is the most abundant protein of the virus particles; it has an important role in the morphology, replication, assembly and budding of the IAV (Liu et al., 2014). The M2 protein is necessary for the uncoating of the virus during the initial stage of infection (Kitikoon et al., 2009). The viral polymerases play a critical role in the replication and transcription of the viral genome (Gabriel et al., 2008). Another protein of interest is the PB1-F2 protein; it is associated with IAV virulence and among other viral factors, enhances lung inflammation during an IAV infection (Chen et al., 2001). The PB1 segment has the potential to alter the IAV pathogenicity, especially with reassortment between human and non-human reservoirs (Alymova et al., 2014).

The nomenclature used for IAV isolates contains five elements; 1. A description of the antigenic type of the virus; 2. The host of origin; 3. Geographical origin; 4. A unique identification code assigned by the detecting laboratory and; 5. Year of isolation. The isolate can further be classified by the antigenic description of HA and NA subtype, e.g. A/swine/Ohio/1/2015(H3N2) (WHO, 1980).

**Influenza A virus evolution**

IAV are known to evolve through two different methods. The first method is genetic drift; this is a slow accumulation of mutations within the viral genome. Over time these base pair changes can lead to changes in the amino acid structure of the proteins.
The second is genetic shift; this occurs when entire segments of the genome are exchanged between viruses during dual infection of a host cell by multiple IAV, resulting in a rapid reassortment of IAV gene segments. These large changes in the genome can lead to novel protein assortments (antigenic shift). When successful reassortment occurs (i.e. functional virus is formed), a novel IAV can be introduced to the host population. Depending on the resulting antigenic properties, the host may have no previous exposure to this newly created strain of IAV, there will be little to no standing immunity in the potential host groups. This lack of immunity and pathogenicity of IAV can lead to large outbreaks (Ma, 2009b).

*Influenza A virus replication*

As an enveloped virus, IAV’s bind to host cells via transmembrane glycoproteins and enters through receptor-mediated endocytosis. Once the vesicle has undergone acidification, the viral membrane fuses with the membrane of the vesicle and the eight viral nucleocapsids are released into the cytoplasm. In the nucleus of the cell viral RNA is copied and, from the messenger RNA, the 11 proteins that comprise IAV are produced. HA, NA, and M2 proteins are transported to the surface of the cell and become incorporated into the plasma membrane. Progeny nucleocapsids associated with the M1 protein and NEP protein then attach to regions of the plasma membrane containing HA, NA and M2 glycoproteins. Virion assembly is subsequently completed as the newly formed IAV virions bud off from the host plasma membrane (Flint et al., 2009). While all of the IAV proteins are important for the function of the virus, the surface proteins and viral polymerase are the determinants of the viral host range. Of the surface proteins, HA in particular is an important determinant of host range, since it binds to the sialylated host
cell receptors (Gagneux et al., 2003). These sialylated receptors are glycosylated oligosaccharides which display sialic acid (SA) residues on the host cell. The SA residues are characterized by the linkage to glycans as either SA α2,3 or SA α2,6 (Kuchipudi et al., 2009). Sialytransferases mediate the SA residues in both a cell- and species-specific manner. (Gagneux et al., 2003). SA receptors play a large role in the determination of host range for an IAV. Additionally the interaction of the viral polymerase with the nuclear import machinery during infection and replication of IAV in the host cell has been shown to be an important determinant of host range (Gabriel et al., 2008).

Influenza A virus hosts

Influenza A virus affects a wide range of host populations and species, such as human, avian, swine, equine, and marine mammals. Wild birds, particularly waterfowl and shore birds, are a natural reservoir for IAV (Webster et al., 1992). Uniquely in these avian species, IAV is an enteric infection. Viral replication occurs preferentially in the cells lining the intestinal tract, normally without causing signs of disease, and virus is shed in high concentration in the feces (Webster et al., 1978). The spread of IAV has been associated with the migration pattern of these avian populations (Slemons et al., 1974). Wild bird lineage IAV have been implicated in outbreaks of influenza in domestic poultry (Horimoto et al., 1995) and in mammals, such as humans (Sun & Liu, 2014, Claas et al., 1998), seals (Geraci et al., 1982), whales (Hinshaw et al., 1986), and swine (Scholtissek et al., 1983). While these instances have occurred, the cross-species spill-over is often self-limiting and rarely maintained in the new host (Ma, 2009a, Vincent et al., 2008). Zoonosis is the cross-species spillover of a pathogen into humans from other animals. Typically in mammals, IAV infections results in respiratory disease with viral
replication occurring in the trachea and lungs (Ito & Kawaoka, 2000, Suzuki et al., 2000). Recent work has suggested that New World bats exhibit a unique reservoir model for IAV (Tong et al., 2012). Along with the striking amount of diversity found in the IAV genome from isolates recovered from bats, analyses of the structure and function of HA and NA indicates that sialic acid is not the attachment or release substrate for IAV in bats (Tong et al., 2013).

Host response to a respiratory influenza A virus infection
Once the IAV enters the host, the body reacts with two different immune responses. First the innate immune response is triggered. IAV is detected in host cells via pattern-recognition receptors (PRRs) which target viral RNA. PRRs are tool like receptors (TLRs), the NOD-likereceptor family pyrin domain containing 3 (NLRP3) protein, and retinoic acid inducible gene-I (RIG-I) (Pang & Iwasaki, 2011). TLR7 attaches to the single-strained RNA. TLR3 and RIG-1 binds to double-strained RNA, signaling for the creation of type I interferons and proinflammatory cytokines to begin. (Alexopoulou et al., 2001, Heil et al., 2004, Lund et al., 2004). Both IFN-β and IFN-α interferons inhibit host cell protein production and limit virus replication. Type I interferon starts additional antiviral activity, such as prompting interferon stimulated genes and dendritic cells for antigen presentation of CD4+ and CD+T cell. (Kreijtz et al., 2011). The cytoplasmic complex NLRP3 imflammasome is associated with immunity against IAV. Once activated NLRP3 will produce IL-1β, a cytokine, which assist in the expansion of antigen-specific CD4+ T cells (Acosta-Rodriguez et al., 2007, Ben-Sasson et al., 2009, Ichinohe et al., 2010).
In the lungs, IAV infected host cells are phagocytosed by macrophages (Kim et al., 2008). They also produce nitric oxide synthase 2 and tumor necrosis factor alpha which causes the pathology associated with IAV damage to host tissue (Jayasekera et al., 2006, Peper & Van Campen, 1995). Dendritic cells are the primary antigen-presenting cell in IAV infection. They detect and opsonize virions and apoptotic bodies from IAV infected cells, and then migrate to present IAV derived antigens to T cells for activation. Dendritic cells play a number of other roles in the immune response, such as exerting cytolytic activity and helping to form bronchus-associated lymphoid tissue (GeurtsvanKessel & Lambrecht, 2008, Hintzen et al., 2006). Natural killer cells recognize antibody-bound IAV infected host cells and lyse them, limiting spread of the virus (Kreijtz et al., 2011).

The second immune response is the adaptive, which can be further subdivided into humoral immunity and cellular immunity. Humoral immunity is the virus specific antibody response, antibodies to the surface glycoproteins (HA and NA) are important for protective immunity against IAV (Gerhard, 2001). HA specific antibodies can either be developed towards the variable head region or the conserved stem region of the glycoprotein. The head region antibodies provide protection against the subtype of HA. The stem region of HA, though it tigers a weaker humoral response, has been shown to provide universal protection across subtypes (Ekiert et al., 2009). NA specific antibodies do not directly neutralize the virus but limit spread by inhibiting enzymatic activity (i.e. not allowing the NA to release the newly formed virion from host cell) (Lamb, 2007). Additional NP specific antibodies may provide protection against IAV infection because NP is as an important target for protective T cells (Carragher et al., 2008). IgA, IgM, and
IgG are the main antibody isotypes in the IAV-specific response. IgA are produced and provide local protection in the mucosal lining of the respiratory track (Mazanec et al., 1995). IgM are the hallmark of primary infection as they initiate complement mediated neutralization of IAV and IgG provide long-term protection against IAV (Murphy et al., 1982, Fernandez Gonzalez et al., 2008).

Cellular immunity response begins with IAV infection. Cytokines are produced to promote immunity (T-helper cells, CD4+ cells) and infected cells are targeted by cytotoxic T-lymphocytes (CTL). Post-infection CTL have been found to remain in the body, circulating and located in the lymphoid organs and these memory CTL show a high degree of cross reaction between IAV subtypes and can function in controlling subsequent IAV infections (Kreijtz et al., 2011). In pigs, IgG antibodies can be found 7-10 days after infection and peak at approximately after 2-3 weeks post challenge (Heinen et al., 2000, Van Reeth et al., 2006). After IAV infections are cleared, strain specific immunological memory is established, which results in a quicker and stronger immune response to homologous IAV challenges, and in some cases cross protection against different subtypes can be provided (Van Reeth & Ma, 2013).

Vaccinations against IAV stimulate the swine immune response, initiating antibody production. There are three vaccination types currently approved for use in swine, first being conventional vaccine containing multiple inactive IAVs. These vaccines provide protection via antibody response will cover identical or very similar IAV to the vaccine strains (Thacker & Janke, 2008). Second, alphavirus-like replicon particles containing RNA encoding for selected HA genes are used to induce antibody production and a cell-mediated response in swine (Bosworth et al., 2010). Third,
autogenous vaccines, like conventional, is also use inactive IAV (Romagosa et al., 2011). However, the isolates used to produce autogenous vaccines are isolated from the swine herd that will receive the vaccination. The effectiveness of all of three IAV vaccinations methods are challenged by the rapid evolution of IAV because strains used in vaccines may quickly fall out of date from strains encountered in the field. Modified live vaccines show promise for inducing broader and more robust protection against IAV in swine (Richt et al., 2006) but currently no such vaccines are commercially available for swine.

1.2 History of influenza A virus in humans

In humans, IAV infection is localized to the respiratory track. The classic signs of disease, coughing and fever, usually occur within 48 hours of onset of headaches, body aches, chills, fatigue and nausea (Monto et al., 2000). On average in the United States, 226,000 hospitalizations and 36,000 human deaths occur annually due to seasonal influenza (Smith, 2006). It is estimated that the total economic burden caused by annual influenza epidemics in the United States is $87.1 billion (Molinari, 2007).

There have been four major pandemics that have resulted in high mortality and disease in humans. Pandemics are the result of a novel IAV arising in the human population, which has the capability to cause disease and spread easily between humans on a global level (Kilbourne, 2006). The 1918 Spanish Influenza (H1N1) pandemic resulted in 20-50 million deaths worldwide (Tumpey et al., 2005), the 1957 Asian Influenza (H2N2) resulted in 1-2 million deaths globally (Kilbourne, 1959), the 1968 Hong Kong Influenza (H3N2) caused 0.7 million deaths globally (Viboud et al., 2005), and the 2009 Swine Flu pandemic (H1N1) caused infection in more than 214 countries with over 18,000 human deaths (Türel et al., 2014). Additionally, there have been three
pandemic threats, which have not fulfill the definition of a pandemic as described above yet demonstrated potential for similar impact: the 1976 Fort Dix Swine Flu, the 1977 Russian Flu and the 1997 Avian Flu (Kilbourne, 2006). To help understand the role and potential threat that IAV poses to the human population, a closer look at the epidemiological and molecular differences between the major IAV pandemics in the last century is needed.

**The 1918 Spanish Influenza pandemic**
This IAV strain abruptly erupted in September of 1918 into a global pandemic after a smaller outbreak earlier that year. Communities experienced a morbidity of 25-40% with the vast majority of cases being self-limiting. Unlike seasonal IAV, morbidity due to the 1918 pandemic was unusually concentrated in a younger age group (Linder, 1943, Marks, 1976), with children under the age of fifteen having the highest rates of IAV infection (Jordan et al., 1927). In fact, greater than 99% of the excess IAV-related deaths in 1918 occurred in those under the age of 65 (Simonsen et al., 2000). The clinical signs of the 1918 virus were similar to those typically associated with IAV infections, such as fever, coughing and congestion. Pathologically, the damage was contained mostly to the respiratory tract (Wolbach, 1919). Secondary bacterial infections played an important role in the devastating global morbidity that resulted from this pandemic. Where exactly the 1918 pandemic originated is a debated topic; arguments are made for avian lineage and for swine lineage IAV. There are reports of IAV outbreaks occurring in the American Midwest pig populations first and then in human (Shope, 1931). However, epidemiological evidence of documented swine outbreaks in Russia and China suggest that the virus movement was a reverse zoonosis occurrence from humans into swine
(Kilbourne, 2006). Supporting this theory is the molecular evidence relating to the structure of the recovered 1918 HA gene. The 1918 HA sequence is more closely related to the avian lineage HA sequence than it is to any other mammalian HA sequence. The recovered HA gene has many of the features associated with avian HA genes, such as similarity in amino acid that are targets of the immune system, and the presence of only four avian glycosylation sites versus the typical five found in modern human HA genes. However, despite these similarities to avian HA gene, phylogenetic analysis continues to place the 1918 virus within the mammalian clade (Reid & Taubenberger, 2003).

**The 1957 Asian Influenza pandemic**

The second influenza pandemic of the 20th century began in February of 1957. Cases were first reported in the Yunan Province of China and swiftly spread, leaving a path of extensive disease throughout the country (Pyle, 1986). By April these IAV reached Hong Kong and continued rampaging into Singapore, Japan and Taiwan. In August 1957 this IAV was well established as a global disease (Fukumi, 1959). This pandemic exhibited clinically typical IAV disease with the young and old experiencing the highest morbidity (Nicholson, 1992). It was highly infectious; 40-50% of people exposed were affected by the pandemic IAV (Potter, 2001). Secondary infection complications, as in the 1918 pandemic, played a role in increasing the number of deaths associated with the 1957 pandemic virus to 86,000 people in the United States (Nicholson, 1992, Morens et al., 2008). The IAV strain responsible for this pandemic was determined to be an H2N2 subtype that was the result of a reassortment of human and avian lineage IAV (Scholtissek et al., 1978a).
**The 1968 Hong Kong Influenza**

The first H3N2 pandemic of the 20th century was initially isolated in Hong Kong in July of 1968 (Cockburn *et al.*, 1969). The pandemic occurred in two waves between 1968-1970. The first (1968/1969) resulted in high mortality in North America, but relatively milder death rates in Europe and Asia (Simonsen *et al.*, 1998). The movement of the disease was described as a smoldering spread outside of the United States. This is thought to be the result of residual antibody protection to the N2 protein from the prior H2N2 pandemic. However, by the second wave (1969/1970) the N2 had changed enough that prior protection afforded by N2 cross-reaction was no longer effective and an opposite pattern was observed with the United States now having a lower mortality relative to Europe and Asia (Viboud *et al.*, 2005, Simonsen *et al.*, 1998). To place this into perspective, in the United States, an estimated 70% of influenza-related deaths for the Hong Kong pandemic occurred during the first pandemic season (1968/1969); in Canada, 57%; in England, 30%; and, in Australia, 31% (Viboud *et al.*, 2005). Interestingly in the United States a higher mortality in those 65 years of age and older was observed during the first wave (Cockburn *et al.*, 1969). Like the 1957 pandemic, the 1968 Hong Kong pandemic virus is thought to be the result of reassortment between avian and human linages of IAV (Scholtissek *et al.*, 1978a).

**The 1977 Russian Flu**

While the 1977 Russian Flu (H1N1) was not as devastating as the previously mentioned pandemics, with typical clinical symptoms and mild disease, it has a number of unique characteristics that bring it up as a topic of interest. The first reported case happened in May of 1977 in northeastern China. This epidemic was almost completely restricted to the those 25 years of age or younger (Kilbourne, 2006). It was soon realized
that this epidemic was a re-emergence of an H1N1 virus that circulated in the 1950s (Scholtissek et al., 1978b), and was preserved laboratory in a laboratory until it was accidentally released into the public in 1977 (Zimmer & Burke, 2009). This also represents the first time that two IAV stains were circulating in the human population at the same time. Previously with each emergence of a new pandemic stain, the existing seasonal endemic IAV subtype was driven out of the human population. However in the 1977 Russian Flu outbreak, both H1N1 and H3N2 subtypes continued to co-circulate in the populace. This is thought to be due to the presence of two different immunity statuses within the human population; those older than 25 years of age with previous exposure to H1N1 and those younger than 25 who did not have any immunity to the H1N1 subtype (Kilbourne, 2006). This co-circulation of multiple subtypes in the human population has continued since 1977 and remains the case in the human population today.

**The 2009 Swine Flu pandemic**

Starting in Mexico April of 2009, a novel H1N1 (H1N1pdm09) IAV began its spread into the most recent influenza pandemic. By May of 2009, the H1N1pdm09 IAV had outcompeted the circulating seasonal H1N1 and H3N2 in the human population (CDC, 2010b). Similar to the 1918 pandemic, children and young adults were at higher risk for infection with the 2009 pandemic then people over 65 years of age (Jain et al., 2009). The 2009 pandemic was a triple reassortment of human, avian and swine lineage IAV (Shoham, 2014). This new virus was a combination of segments most closely related to North American swine lineage H1N1 and Eurasian lineage swine origin H1N1 IAV thus giving this pandemic the “Swine Flu” name. Death due to this pandemic was far less than the 1918 pandemic, due to developments of modern medicine. Secondary bacterial
infections that devastated the population with IAV during the 1918 pandemic could now be treated and supportive care more readily offered (Morens et al., 2010). Over 214 countries and territories reported cases of H1N1pdm09 and over 18,400 confirmed deaths. The true impact of the 2009 pandemic is likely substantially larger (CDC, 2010a).

1.3 The importance of a swine host

Pigs have been coined “the mixing vessel” for IAV, as novel IAV strains have habitually been found in the swine population (Scholtissek, 1990, Ma, 2009a). Recalling the sialylated host cell receptors that allow for viral entry into the host cell, the swine respiratory tract encompasses SA α2,3 and SA α2,6 receptors that readily bind with IAV. Historically, avian lineage IAV preferentially binds to SA α2,3 receptors and mammalian lineage IAV bind to SAα2,6 receptors. Figure 2 shows the distribution of these SA receptors between swine, human, ferret and avian hosts. Having the ability to bind with both types of receptors, dual infection in pigs has served as a pathway for genomic reassortment to occur (Ma, 2009a). However more recent work has shown that pigs are not unique in their distribution of receptor types. Humans and some avian species, such as quail, have been found to have a presence of both SA α2,3 and SA α2,6 receptors (Wan, 2006, Shinya, 2006). While avian lineage IAV, specifically the H5N1 subtype, has been shown to effectively replicate in the human lung, transmission among humans has been low. This poor transmission rate has been hypothesized as a result of SA receptor distribution in humans. Specifically, SA α2,3 receptors, which avian lineage IAV prefer, are located deep in the human respiratory tract which limits the ability to expel viral progeny (Claas et al., 1998). Some studies suggest that in the respiratory distribution of SA cell receptors alone, human and swine have the same potential to act as a host for
reassortment of IAV (Nelli et al., 2010, Van Poucke et al., 2010). However, the respiratory track of the pig is more optimal for co-infection occurrence and for release of viral progeny for transmission. Thus swine can serve as an adaptive pathway for avian lineage IAV transmission to humans (Ma, 2009b, Ma, 2009a).

**Figure 1.2**: The distribution of SA α2,6 (red stars) and SA α2,3 based on Maackia amurensis agglutinin (MAA)-I (blue stars) and MAA-II staining (yellow stars), where star size illustrate receptor abundance (de Graaf & Fouchier, 2014).

1.4 History of influenza A virus in swine

**North American pigs**

Influenza was first identified as a disease in swine during the Spanish influenza pandemic of 1918-1919, when millions of pigs in the Midwestern United States became ill with the respiratory diseases and thousands died (Easterday, 2003). IAV was later isolated from pigs in North America in 1930 (Shope, 1931). This classical H1N1
(cH1N1) was virtually the only IAV circulating in the United States swine population for 68 years. Annual outbreaks occurring in the winter months were observed and it was frequently associated with other agents of respiratory disease.

New influenza outbreaks began occurring among swine in the United States during 1998. Starting in North Carolina, a swine farm observed influenza-like-illness among their pigs. Later that same year, Minnesota, Iowa and Texas all reported similar cases of IAV outbreaks in swine populations. Once the viruses were isolated, genetic analysis found them to be reassorted H3N2 IAV. The North Carolina isolate from earlier that year was a double reassortant (dH2N3) with human lineage (HA, NA, PB1) and swine lineage (NS, NP, M, PB2, PA). The isolates recovered from the later outbreaks showed a triple reassortment (tH3N2) with human lineage H3N2 (HA, NA, PB1), swine lineage cH1N1 (NS, NP, M) and avian lineage (PB2, PA) (Zhou et al., 1999). By the fall of 1999, the tH3N2 became widespread as an endemic IAV in the North American swine population (Karasin, 2000b). The six internal genes in this assortment of human, avian and swine lineage IAV described above is referred to as the triple reassorted internal gene (TRIG) constellation. The tH3N2 and cH1N1 continued to evolve as they co-circulated in the swine populations. With the mix of additional HA genes from a human seasonal H3N2, three distinct phylogenetic clusters developed for the H3 segment and were classified as I, II, and III (Gramer et al., 2007). An additional cluster IV has since evolved for the H3 segment and become widespread as the common H3 type (Christopher et al., 2006). This cluster IV was first classified into two antigenic subclusters, H3N2-α and H3N2-β (Feng et al., 2013). Today in North American swine,
Cluster IV H3 IAVs have been subclustered into six clades classified at A-F (Kitikoon et al., 2013, Lewis et al., 2014 (Anderson et al., 2015)).

In 1999, H1N2 also made its first appearance in North American swine populations. This reassortment between cH1N1 (HA) and tH3N2 (NA) contained the TRIG internal gene segments (Karasin, 2000a). Sustained isolations of the H1N2 demonstrated that it became a prevalent IAV subtype in the swine population (Karasin et al., 2002). Other mixing of TRIG with cH1N1 (HA and NA) lead to a resorted H1N1 (rH1N1) being introduced into the swine population (Webby et al., 2004). During 2003 a new H1N2 subtype of human lineage was recovered from swine farms in Canada. The surveillance study also found a uniquely resorted H1N1, that was cH1N1 with a human lineage PB1 (Karasin et al., 2006).

In 2009, a triple reassortment of human, avian and swine lineage IAV took place, the gene segments were cH1N1 (HA, NP and NS), tH3N2 (PB2, PA, PB1) and Eurasian ‘avian-like’ H1N1 (NA and M). The result was the “Swine Flu” pandemic of 2009, the H1N1pdm09 caused similarly large outbreaks in swine populations (Jian-Hua et al., 2012, Shoham, 2014, Zimmer & Burke, 2009). After which the H1N1pdm09 subtype became firmly established as an endemic virus in the pig populaces. Further reassortment has led to the introduction of the H1N1pdm09 internal genes into most IAV circulating in the North American swine population. Recently work by Anderson, et al. have identified a new cluster γ-2, which is thought to have been circulating in the swine population since 1995, showing a need for a further expansion in swine surveillance (Anderson et al., 2013). H1 diversity in the United States can be classified into seven distinct genetic groups. These groups cluster into α- (cH1N1), β- (rH1N1), γ- (reassortments between
cH1N1 and tH3N2), γ-2, δ1, pdm09 and δ2 (H1N2 and H1N1) cluster (Lorusso et al., 2011, Vincent et al., 2009). Figure 3 shows a visual depiction of IAV strain evolution in the North American swine populations.

**Figure 1.3**: Evolution of the IAV stains in North American swine populations. (Nelson et al., 2011)

*Surveillance for influenza A viruses in the swine population*

Current surveillance in the United States swine population has increased since 2009. Due to this increase in surveillance, a rapid expansion of viral diversity can be
observed over the last six years (Anderson et al., 2013). As seen in Figure 1.4, the number of sequenced swine isolates increased between 2009 and 2012. The three major subtypes H1N1, H3N2 and H1N2 have continued to persist in the population (Anderson et al., 2013). Recent surveillance analyses preformed by Anderson, et al. looked at 1,576 H1, 607 H3, 834 N1, 1,293 N2 and 2,126 M gene segments. Currently there are 7 clusters associated with the H1 gene and identified the following prevalences, 0.17% α-, 3.56% β-, 49.66% γ-, 0.34% γ-2, 37.28% δ1, 4.07% δ2 and 4.92% pdm09. For H3 gene among the 5 clusters I, II, III and IV, only cluster IV was identified. Cluster IV has further been divided into six subclusters 76.68% A, 13.90% B, 2.24% C, 0% D, 3.14% E and 2.69% F. All circulating NA genes in the United States swine were found to be N1 or N2 subtypes. N1 were either classical origin (87.4%) or H1N1pdm09 origin (12.6%). N2 genes were 1998 lineage (6.0%) or 2002 lineage (94.0%). M genes were H1N1pdm09 lineage (90.7%) and North American swine lineage (9.3%) (Anderson et al., 2015).

To date, seroprevalence studies in the United States have shown that commercial swine commonly have antibodies to the three major subtypes, H1N1, H3N2 and H1N2 (Choi, 2002). These antibody levels could be due to either IAV infection or from vaccination for influenza.
Figure 1.4: Sequenced swine influenza A viruses in United States. Submitted to the anonymous and voluntary USDA surveillance system through the NAHLN laboratories from 2009 to 2012. (A) Swine influenza A subtypes (H1N1, H1N2, H3N2, and mixed) identified by year; (B) State participation and swine population (Anderson et al., 2013)

Swine outside of North America

After the 1968 Hong Kong pandemic, the H3N2 subtype was isolated from 139 pigs in Taiwan in 1969. Furthered serological and virological studies found this ‘human-like’ H3N2 subtype in swine populaces in the United States, China and many other countries (Acha & Szyfres, 2003). Interestingly, continued isolation of ‘human-like’ H3N2 among swine populations, after circulation in the human population had decreased, suggest an endemic establishment of this subtype in the some swine populations.

In 1976, over thirty years after its first isolation in the United States, cH1N1 was isolated in Europe. The source was pigs shipped to Italy from the United States (Nardelli
et al., 1978). Antibodies and IAV isolates deemed cH1N1 were found in pig populations around the world, appearing in North American, South America, Asia, Africa and Europe (Brown, 2000).

In 1979, an H1N1 that was antigenically distinct from the cH1N1 previously seen in swine population emerged to co-circulate with the ‘human-like’ H3N2 subtype among the European swine. This ‘avian-like’ H1N1 soon replaced the cH1N1 among European pigs. This is thought to be the product of an avian lineage H1N1 moving into the swine population (Marozin et al., 2002). This Eurasian ‘avian-like’ H1N1 would provide the N1 and M genes in the triple reassortment that lead to the H1N1pdm09 (Shoham, 2014).

In 1980, a genetically resorted IAV strain was isolated in Japan from swine (Acha & Szyfres, 2003). The H1N2 isolate was a reassortant, having the HA of cH1N1 and the NA that was similar to the H3N2 strain isolated from human cases. This subtype became wide spread in the Japanese swine population.

The reassortment of the ‘avian-like’ H1N1 (NS, NP, M, PB1, PB2 and PA) with the co-circulating ‘human-like’ H3N2 (HA and NA) in European swine occurred during 1984. The product was a reassorted ‘human-like’ H3N2 (r’human-like’H3N2) (Castrucci et al., 1993). During 1987, a new H1N2 constellation was isolated. It contained gene segments from r’human-like’H3N2 and ‘avian-like’H1N1. However, it did not spread through the swine populations (Gourreau et al., 1994). In the early 1990s, a genetically and antigenically distinct H1N2 IAV strain emerged in the United Kingdom. It contained the human H1N1 (HA) and r’human-like’H3N2 (NA) and was termed resorted H1N2 (rH1N2). It has since, became the predominant subtype in United Kingdom pig populaces and spread in Europe (Marozin et al., 2002, VanReeth, 2003). In 2009 the H1N1pdm09
moved into the European swine population. This H1N1pdm09 became established in the population and through reassortment, its genes were introduced into the circulating strains (Harder et al., 2013, Simon et al., 2014). A similar introduction of the internal H1N1pdm09 gene segment into endemic swine lineage IAV strains was observed in China (Liang et al., 2014).

1.5 Risk factors for influenza A virus in swine
Most studies looking at risk factors for IAV in swine are in the commercial swine herd setting. A study in Ontario swine herds found elements associated with sow-herd IAV positivity were pig/farm density at different geographic levels, an external source of breeding pigs, number of animals on site, and decreasing proximity to other barns. Additional higher-parity sows had higher odds of seropositivity, however there was significant random variability of this factor between herds. In finishing-herds the odds of IAV positivity increased for every 1,000 pigs (Odds Ratio (OR) = 4.44, 95%, confidence interval (CI): 1.90-13.07, \( p <0.01 \)), high pig farm density in 0.1 farms/km\(^2\) (OR = 1.41, 95% CI: 1.00-2.04, \( p =0.02 \)) and finishers only status of farm (OR = 0.11, 95% CI: 0.01-0.62, \( p <0.01 \)). It was observed that finisher herds were IAV-positive only if the source sow herds were positive, though IAV-positive sow sources could produce IAV-negative finisher herds. (Poljak et al., 2008). A larger study in Spain looked at risk factors associated with IAV prevalence in swine at 98 farms. From the 91 farms that had IAV seropositive animal three risk factors were found to be associated with seroprevalence: increase replacement rates in pregnancy units for sow herds, existence of open partitions between pens, and uncontrolled entrance to the farm for finisher herds (Simon-Grife et al., 2011). There is much support that increasing density and herd size are associated risk
factors for IAV in pigs (Gardner et al., 2002, Maes et al., 2000, Yus et al., 1992). Corzo et al., assessed IAV risk factors for detection of IAV in growing pigs. Associated farm-level characteristics included gilt source (offsite OR=0.25, other OR=0.17), farm type (farrow-to-finish (OR=3.05); nursery (OR=16.69), pig flow (all-in, all out (OR=0.31) by barn, (OR=0.35) by site), environmental temperature and wind speed (Corzo et al., 2014). A supporting study for increased population as a risk factor for IAV in pigs outside of the commercial setting was conducted at 40 agricultural fairs in the United States. It was found that the adjusted odds of having an IAV infected pig at the fair were 1.27 (95% confidential interval (CI): 1.04-1.66) higher for every 20 pig increase in the size of the swine population at the fair (Bowman, 2014).

1.6 Clinical signs of influenza A virus in swine

While clinical signs due to IAV can be variable they are typically characterized by loss of appetite, lethargy, dyspnea, fever, nasal discharge and (LeFloc'h, 2014, Vincent et al., 2008). High morbidity with low mortality is generally observed within a herd and typical clinical signs are often demonstrated from 25–100% of the infected individuals in the herd (Brown, 2000, Vincent et al., 2008). IAV in swine has proved to be subclinical in many cases; swine at 83.3% of the agricultural fairs with IAV infected pigs showed no clinical signs of IAV (Bowman, 2012, Pomorska-Mól, 2014). IAV is a component of the porcine respiratory disease complex (PRDC), which is the interactions of viral, bacterial components and adverse management conditions that result in the manifestation of respiratory disease in pigs (Brockmeier SL, 2002). IAV infection is thought to make pigs more predisposed to bacterial pneumonia due to damage of the mucociliary apparatus and decreased macrophage function (Brockmeier SL, 2002). On
sow farms, the introduction of IAV into the herd can produce abortions due to systemic illness, not primary infection of the fetuses (Choi et al., 2002). There have been multiple estimates on the economic impact of IAV in the commercial swine industry. Donovan, comparing an affected to unaffected pig flow, found a 2.9 % greater wean-to-finish mortality in affected pigs (Donovan, 2005). In 2007 another group found that IAV infection in pigs led to 0.04-0.05 decrease in feed efficiency and a 2.5 % nursery-finish mortality (Holtkamp, 2007). Others estimate the additional cost of IAV infections to be $10.31 per market hog (Donovan, 2008), and $3.23 per pig through closeout cost (Dykhuis, 2012).

1.7 Transmission of influenza A virus in pigs

Transmission between swine occurs by direct or indirect contact with respiratory secretions from pigs infected with IAV (Brown, 2000). IAV is shed in the mucosal secretions of pigs starting 1 day post infection and continues to be shed 5 to 7 days post infection with or without clinical signs (VanReeth, 2003). Four days after comingling with experimentally infected pigs, naïve pigs begin to shed infectious virus as well (Lange, 2009). Transmission of IAV can occur through indirect contact such as fomites and aerosolized droplets has also been proven (Allerson et al., 2013, Tellier).

1.8 Sample collection methods for influenza A virus in swine

The gold standard for sample collection for IAV in swine is the nasal swab method (Van Reeth, 2012). This procedure uses a synthetic fiber-tipped swab to collect nasal mucosal secretions and surface epithelial cells. Larger swine are restrained using a snare over their upper jaw and the swab is inserted into each nasal cavity and then placed into a viral transportation media until testing can occur. Viral transport media helps to
keep IAV stable during the time period between collection and testing (Spackman et al., 2013). Another method used for collecting the nasal mucosal secretion is snout wipes. Here a sterile cotton gauze pad is used to wipe across the pig’s snout, ensuring that the gauze pad makes contact with the external nares (Edwards, 2014). The wipe is then placed in a viral transport media and stored for testing. Snout wipes have an advantage of not requiring restraint of the pig and require less time to collect in the field, however snout swipes are a less sensitive sampling method than nasal swabs. A comparison by realtime-PCR found snout wipes to detect 82.9% (95% CI: 76.1-88.1) of the IAV positive animals that were found via nasal swabs (Edwards, 2014). While less sensitive, the snout wipes offer an advantage in ease of physical collection that make this test a useful method for sampling large numbers of individual pigs. The presence of IAV in swine herds has also been detected through oral fluids (Ramirez, 2012). Oral fluid samples are most often used as a method for herd level detection of IAV in commercial settings. A cotton rope is hung in a cohort of pigs, thus allowing the pigs to chew on the fibers. After a period of time the oral fluids accumulated on the rope are decanted and stored for sampling. While this method works for herd detection, collection of oral fluids from individual pigs is not always feasible since the pigs are required to chew on the fibers and requires a longer period of time to collect (Decorte et al., 2015). Another common sampling method is blood and sera samples. Blood can be taken from the pigs on farm or during slaughter for serological testing.

1.9 Diagnosis of influenza A virus in swine

Since the clinical signs of IAV in swine are non-specific and often even subclinical, testing is needed to diagnose IAV. Two types of testing may be performed
depending on the sample(s) collected from the animal, indirect testing and direct testing. Indirect testing can determine the presence of antibodies to IAV (CDC, 2009). The four methods are typically used to accomplish this are enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition assay (HI), agar gel immunodiffusion (AGID) test, and indirect florescent antibody (IFA) test (Pedersen, 2008, Lee et al., 1993, Peng et al., 2007, Nutter et al., 2012). In an ELISA, antigens for IAV are attached to a surface. This surface is then exposed to the collected sample; and if IAV antibodies are present they will bind to the antigens attached to the surface. A secondary antibody that is conjugated to an enzyme will be added; this enzyme will interact with a substrate to create a color change for detection (Lee et al., 1993). When used for the detection of IAV in swine, the commercially available kit for avian lineage IAV that targets the NP protein antibodies is used (Ciacci-Zanella et al., 2010). HI assay, one of the original tests for IAV, also tests for the presence of antibodies within the sample. Influenza A virus, Newcastle disease, fowl plague, variola virus and mumps virus are all examples of hemagglutinating viruses (Briody, 1946). These viruses will bind to the receptors on blood cells causing hemaglutination. In the presence of antibodies this viral binding is inhibited and hemaglutination will not be observed. If paired sera samples can be obtained, serial serological testing will establish a retrospective diagnosis of IAV through antibody levels. AGID test involve an antigen antibody reaction in a agar gel to detect IAV antibodies (Peng et al., 2007). IFA uses fluorescently bound antigens to determine the presence or absence of IAV antibodies (Nutter et al., 2012)
Conversely real-time RT-PCR (rRT-PCR), virus isolation, visualization, antigen testing (rapid influenza diagnostic test (RIDTs) and immunohistochemistry (IHC) are examples of direct testing methods for IAV (Ginocchio, 2009). The current diagnostic method of choice for many laboratories is rRT-PCR for the detection and subtyping of IAV (Zhang, 2014b). RNA is extracted from the samples, translated into cDNA and run on an rRT-PCR assay which targets segments of the matrix gene for IAV. This test is sensitive and specific for IAV, however it does not differentiate between active virus and residual RNA. Virus isolation for swine lineage IAV most commonly occurs in cell culture using Madin-Darby canine kidney (MDCK) cells. While embryonated chicken egg inoculation is considered the “gold standard” for avian lineage IAV isolation and propagation, MDCK cell culture has proven more effective for swine lineage IAV recovery, since MDCK cell have an equal distribution of SA α2-6 and SA α3-6 receptors and are more cost effective (Zhang, 2014a, Bowman, 2013). Electron microscopy can be used to visually identify influenza viruses based on their size, shape and structure (Compans & Dimmock, 1969). The final example of direct testing is the use of antigen detection. RIDTs, are a manufactured kit for the detection of IAV. The test strip membrane contains specific antibodies used to capture the NP and generate test results in 10-15 minutes (Balish, 2014). RIDTs are highly specific, however reports have shown that RIDT analytical and clinical sensitivity varies (Weinberg, 2005). IHC uses specific antibodies tagged with enzymes to bind to the antigen within a tissue sample. Enzymes when exposed to substrate will then undergo a reaction, producing a color change indicating the presence, location and abundance of the IAV in the tissue.
1.10 Commercial swine in the United States

Historically in the United States, swine production has occurred in the “Corn Belt” states of Iowa, Illinois, Indiana and Minnesota. Swine are generally weaned at 2-4 weeks of age when they are then moved into a nursery, grower or a wean-finish building depending on the methods used. In the 1970’s the swine industry started to undergo a shift, with large production facilities being opened in the Southeast (North Carolina) and Southwest (Oklahoma). The cost of transporting pigs up to the Midwest for fattening and slaughter was more economic than transporting feed down to the southern facilities. Thus the continuous large scale movement of pigs in trucks from the South to the Midwest (swine-flows) began. In 2011 a study was conducted to see if there was any correlation between IAV movement throughout the United States and the establishment of the “swine-flows”. Nelson et al. found that novel IAV subtypes originated in the Southern pig facilities and were disseminated into the Midwest swine populations following the transportation routes of swine. While the Midwest was not traced as a source of IAV it most likely provides a reservoir for IAV to co-circulate and for viral reassortment to take place (Nelson et al., 2011). In 2012, a national inventory found 66,026,785 pigs in the United States (USDA, 2012). The top ten states and their production of pork were Iowa (19,200,000 head), North Carolina (9,600,000 head), Minnesota (7,300,000 head), Illinois (4,300,000 head), Indiana (3,650,000 head), Missouri (3,100,000 head), Nebraska (3,100,000 head), Oklahoma (2,300,000 head), Ohio (2,010,000 head) and Kansas (1,810,000 head) (USDA, 2012).
1.11 Exhibition swine in the United States

The North American show pig industry is comprised of a diverse mixture of open class shows, breeding shows, “jackpot shows” and educational youth programs such as FFA and 4-H. Exhibition swine used for FFA or 4-H projects are typically obtained during the spring and shown during the summer at agricultural exhibitions in the Midwest. However, the swine show circuit takes place year round. The annual number of 4-H swine program participants in the United States between 1996 and 2003 have ranged from 140,000 to 212,000 (Wayne, 2011). If the fact that an exhibitor can show multiple pigs is taken into account, an estimate of the expected number of pigs shown per year is 200,000 (Wayne, 2011). This number does not account for the pigs shown in open class, in breeding shows, or as FFA projects. The National Swine Registry estimates that 1 million swine are involved in the exhibition swine industry (National Swine Registry, personal communication, 2015). Additionally, these pigs are sometimes shown at multiple exhibitions within a year.

In 2005 Wayne et al., conducted a surveillance study focused towards on-farm management practices among 4-H swine exhibitors in Minnesota. Twenty four percent (95% CI, 17% to 32%) of the 4-H participants reported having contact with swine other than their own at least once a week. Other livestock were often found to be raised at the same location as the exhibition swine; of the 77% (95% CI, 69% to 84%) of farms that raised other livestock, cattle (56%), sheep (26%), goats (8%), poultry (28%) and other species (21%) were reported. Commercial swine production at the same location as the exhibition swine occurred at 36% (95% CI, 28% to 45%) of the farms. Among 15 (32%) farms with reported commercial swine production, both commercial and exhibition swine
were raised in the same barn. 4-H responders reported showing their pigs on average 2.5 times during the year (median 1) with one responder reporting attending 35 swine shows.

A study in 2005 at the California State Fair looked at biosecurity and animal movement of the swine projects related to 137 of their exhibitors. On average, animals shown at the California State Fair participated in 3 exhibitions during a 12-month period (range from 1 to 7). The study also found that 787 of the 812 (97%) animals shown at the California State Fair would be returning to the farm after the show. Among farms with returning animals, 33 (26%) would put the animal under quarantine on-farm (Thunes, 2007).

The presence of IAV in swine at agriculture exhibitions has been well documented (Bowman, 2012, Killian et al., 2013, Bowman, 2014, Gray, 2012, Wells et al., 1991). During 2009-2012, surveillance testing for IAV found 22.6% (12/53) of the exhibitions tested had pigs positive for IAV via rRT-PCR and VI. Of the positive fairs, on average IAV could be recovered from 62.9% of the pigs (Bowman, 2012). The adjusted odds of having IAV infected pigs at a fair were 1.27 (95% CI: 1.04-1.66) higher for every 20 pig increases in the size of the swine show (Bowman et al., 2014). Little is known about the frequency of pigs that arrive at exhibitions already infected with IAV, since most testing takes place at the end of the fairs after the pigs have comingled for several days. It is important to note that the genetics and desired qualities for show pigs are greatly different than those found in commercial swine, and that this industry is typically perceived as distinct from the commercial pork industry. There is limited knowledge concerning the interaction that takes place between these two swine populations.
1.12 Zoonotic Movement of influenza A virus between swine and humans

Transmission of IAV between human and swine populations has been documented since the first isolation of swine lineage IAV in a human during 1974 (Smith et al., 1976) and hypothesized far earlier during the Spanish influenza pandemic, when people in close contact with pigs began displaying similar signs of disease as their infected swine herds (Easterday, 2003). A review by Myers et al in 2007 identified 50 cases of swine lineage IAV in humans. These were studied as civilian and military cases separately. Thirty-seven of the 50 were civilians with cases occurring in United States 51.3% (n=19), Czechoslovakia 16.2% (n=6), Netherlands 10.8% (n=4), Russia 8.1% (n=3), Switzerland 8.1% (n=3), Canada 2.7% (n=1), and Hong Kong 2.7% (n=1). The United States cases were located in Wisconsin 31.6% (n=6), Minnesota 15.9% (n=3), Virginia 10.5% (n=2), Texas 10.5% (n=2), Nevada 5.3% (n=1), Iowa 5.3% (n=1), Missouri 5.3% (n=1) and Maryland 5.3% (n=1). The sex distribution of the patients found 62.8% (22/35) were male. The median reported age was 24.5 years. When prior contact with swine was examined it was found that 57.9% (22/38) had recent exposure to pigs, 36.8% (14/38) had no known contract with pigs and 5.3% (2/38) were laboratory workers that had become accidentally infected by the sick pigs with which they were working. The mortality from IAV was 17% (6/37). The majority of cases were H1N1 with 4 cases being H2N3 (Myers et al., 2007).

In 1976 one of the largest outbreaks of swine lineage IAV in humans occurred at Fort Dix. Two hundred and thirty military personal became ill, 13 were hospitalized and 1 patient died. All of the hospitalized cases had previously been healthy males, averaging 18 years of age. The IAV isolated was found to be swine lineage H1N1 was thought to be
brought in by an incoming trainee, and no known contact with swine was documented. Spread of the IAV was limited to the military base personal (Top & Russell, 1977).

In Wisconsin during September of 1988, a previously healthy 32 year old pregnant woman developed swine lineage IAV after attending a county fair where pigs had been reported ill. She died 8 days after being admitted to the hospital for pneumonia. Serological testing of 25 of the swine exhibitors found 19 (76%) were expressing hemagglutination-inhibition titers to swine lineage IAV. Health care workers who had contact with the patient during her hospitalization developed influenza like illness with laboratory evidence of swine lineage IAV infection (Wells et al., 1991). Another case of swine lineage IAV in Wisconsin was reported in 1995. A previously healthy immunocompetent 37 year old woman was diagnosed with swine lineage H1N1 and died 3 days after hospitalization. The patient had previous exposure to ill swine at the farm she was working (Kimura et al., 1998). These cases showed that contact with clinically ill pigs can transmit IAV into a human host and that the swine lineage IAV could be further transmitted from the infected human host into other people.

In 2007, an outbreak of swine lineage H1N1 in the exhibition swine and humans occurred at a county fair in Ohio. Morbidity in swine reached ~100% and two dozen people attending the fair presented IAV symptoms. IAV was isolated from pigs and 2 people (parent and child), isolates were 100% identical between the human and swine cases, suggesting a direct transmission of IAV from pig to person (Killian et al., 2013).

In 2009, a 12 year old boy contracted swine lineage H3N2 after petting multiple apparently healthy pigs at a county fair in Kansas. This case illustrated that pigs do not need to be showing clinical signs for zoonotic transmission of IAV to occur and serves as
another example that agricultural fairs are a location for zoonosis to transpire (Cox et al., 2011). In the winter of 2011, the number of swine lineage H3N2 human cases in the United States started to increase. By the end of the year there had been 12 human cases. This trend in swine lineage H3N2 continued into 2012 with an explosive increase to 309 human cases. The occurrence of swine lineage H3N2 slowed in 2013 with 19 human cases and by 2014 was down to 3 human cases (CDC, 2014c). Most cases of swine lineage IAV have occurred in Indiana (154 cases) and Ohio (110 cases) (CDC, 2014d). The transmission of IAV at agriculture fairs is well documented, with a large amounts of swine variant IAV human cases corresponding to exposure to pigs at fairs (Bowman, 2012, Feng et al., 2013, Bowman, 2014, Wong et al., 2012, CDC, 2012). Estimates for the number of unreported variant IAV cases place thousands of people contracting this zoonotic pathogen in the United States (Biggerstaff et al., 2013).

The interface between humans and animals at agriculture fairs provides a unique opportunity for a mixing of multiple animal species in high density. The risk for zoonosis and reverse zoonosis is increased under these condition. IAV is not the only pathogen present at agriculture exhibits, for example at Colorado fairs, 11 different poultry exhibits were environmentally tested for Salmonella and 10 of the fairs were positive (Pabilonia et al., 2014). Additionally, 106 people were affected by an outbreak of Esherichia coli, which resulted from contact with petting zoo animals at the Cleveland fair, in North Carolina (North Carolina Department of Health and Human Services, 2012). Agriculture exhibitions provide an opportunity for the comingling of a diverse group of pigs and humans (Gray, 2012).
The importance of preventing variant IAV at this exhibition interface has been recognized and a number of mitigation strategies have been put forth by the National Association of State Public Health Veterinarians (NASPHV), National Assembly of State Animal Health Officials (NASAHO), Centers for Disease Control and Prevention (CDC) and National 4-H Headquarters. Individuals at high risk such as children under 5, elders over 65, immunocompromised and those with chronic diseases that could cause complications due to IAV are advised to avoid contact with swine or the environment of swine. Those individuals not at high risk, should avoid or minimize contact with ill swine, practice good hand hygiene and use personal protection if coming into direct contact with ill swine. Swine caretakers have been recommended by CDC to receive seasonal influenza vaccinations to prevent reverse zoonotic transmission on-farms (CDC, 2014b). For exhibition swine, it has recommended that pigs receive vaccinations for IAV and veterinarians should be contacted if swine start to demonstrate clinical signs of IAV infection (CDC 2013, NASAHO, 2014). Other strategies for decrease in IAV in exhibition swine include the implementation of good biosecurity measures, such as isolate and observe swine for ILI for seven days after exhibition attendance, clean and disinfect equipment and decreasing the amount of time exhibition swine spend at fairs (NASAHO, 2014, CDC 2013). However, it is unknown if these practices have been put into place by swine exhibitors.

1.13 Conclusions

Developing a better understanding of IAV in swine populations for the prevention of disease in both swine and human populations is critical for the control of zoonotic transmission and spread. The present assessment of the literature identified two gaps in
the current information on exhibition swine. Firstly, the geographical distribution of exhibition swine and knowledge about how these exhibition swine are raised remains relatively undefined in comparison to our knowledge of the commercial swine industry. Studies that have sought to characterize some of the management practices of the exhibition swine industry have occurred in a limited geographical areas and may not be representative of the exhibition swine population as a whole. Understanding where and how these exhibition swine are raised is important for comprehending the movement of IAV through the exhibition swine population and for making realistic mitigation strategies for controlling IAV in this population.

The second gap in information on exhibition swine lays in the movement of IAV at agricultural fairs. While the presence of IAV among swine at fairs and the correlation between these events and variant cases of IAV in humans has been well documented (Bowman et al., 2012a, Bowman et al., 2012b, Jhung et al., 2013), only a few limited studies have been performed to assess how IAV enters these agricultural exhibitions. Understanding the frequency with which pigs arrive infected with IAV at these fairs will aid animal health officials as they seek to implement mitigation strategies aimed at decreasing human and swine exposure to IAV at these agricultural exhibitions.

Thus the objectives for the present project are: 1. To determine the incoming prevalence of influenza A virus among exhibition swine at agricultural fairs. 2. To characterize the on-farm management practice used for exhibition swine and determine if any management practices are associated with bringing IAV to agricultural fairs.
CHAPTER 2: The prevalence of influenza A virus in exhibition swine during arrival at agricultural fairs.

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2.1 Impacts

- The frequency of influenza A virus isolation from exhibition swine arriving to fairs in the Midwestern United States was low at 1.49% (53/3,547).
- Intra-exhibition pig movement and corraling activities, which are typical fair management practices, likely enhance pathogen transmission during exhibitions.
- Due to the low overall prevalence of influenza A virus in swine at the beginning of fairs, focus should be placed on mitigating influenza A virus spread during swine exhibitions rather than attempting to completely preclude entry of influenza A virus infected swine.

2.2 Summary

The exhibition of swine at agricultural fairs provides a critical human-swine interface that allows for the bidirectional zoonotic transmission of influenza A virus (IAV). Previous IAV surveillance at fairs has documented the regularity with which
swine infected with IAV are detected at the end of exhibitions, but little is known about
the frequency with which swine arrive at fairs already infected with IAV. We sought to
investigate the incoming IAV prevalence among exhibition swine to better understand the
epidemiology of IAV in this unique human-swine interface. In 2014, snout wipes were
collected from 3,547 swine during the first day of nine agricultural exhibitions in Indiana
and Ohio. Samples were screened for IAV using rRT-PCR and positive samples were
inoculated into cultured cells for virus isolation. The overall IAV prevalence, as
determined with virus isolation, was 1.5% (53/3,547) among swine arriving at
exhibitions, with IAV being recovered from swine at 5 of the 9 exhibitions. Within the
fairs with IAV positive swine, the individual exhibition IAV prevalence ranged from
0.2% (1/523) to 10.3% (43/419). Only one IAV subtype was detected per fair at three of
the fairs but the IAV isolates at two of the exhibitions displayed subtype diversity as both
H1N1 and H3N2 were recovered from incoming swine. At two of the exhibitions, a
temporal relationship was observed between the order of the individual swine in sampling
and the associated IAV rRT-PCR results, indicating the fomite transmission of IAV
through common contact surfaces may occur. With the knowledge that a small proportion
of swine arrive at fairs shedding IAV, resources should be directed toward preventive
strategies focused on limiting swine-to-swine transmission during fairs to protect swine
and humans during exhibitions.

**Keywords**

Swine, Influenza A virus, Virus Shedding, Fairs, Prevalence, Animals
2.3 Introduction
Influenza A virus (IAV) is an endemic pathogen in the North American swine population (Vincent et al., 2008). Swine actively infected with IAV may display clinical signs characterized by loss of appetite, lethargy, dyspnea, fever, nasal discharges, and coughing (Vincent et al., 2008). Swine also experience subclinical IAV infections which complicates typical detection, as infected pigs may not display clinical signs of disease (Bowman, 2012, Gray, 2012).

The exhibition swine industry is a diverse mixture of culture, education, and business. Swine are exhibited as 4-H and FFA projects at agricultural exhibitions to expand youth knowledge about agricultural practices. Additionally, swine can be shown at multiple times across multiple locations in exhibitions open to any age competitor throughout the year in the United States. For 2015, the National Swine Registry estimates that 1 million swine are involved in the United States show pig industry (National Swine Registry, personal communication, 2015). Exhibition swine are a relatively small part of the national swine industry as a whole, representing an estimated 1.5% of the total swine population in the United States (USDA, 2012); however, exhibition swine represent the most common direct interface with a large number of humans, outside of the owners and caretakers, when commingled for the competition events.

Swine are considered a potential source of novel IAV for the human population, as swine have demonstrated the capability to serve as “mixing vessels” in which multiple IAVs can reassort (Scholtissek, 1990, Ma, 2009a). When humans are infected with an IAV that typically circulates in the swine population, the infection is classified as “variant” IAV (WHO, 2014). Zoonotic movement of IAV between swine and people is
possible and has been documented most commonly at swine-human interfaces such as agricultural fairs and live animal markets (Jhung *et al.*, 2013, Choi *et al.*, 2014, Shinde *et al.*, 2009).

Cases of IAV transmission between swine and humans at agricultural fairs have been well documented in the United States (Jhung *et al.*, 2013, Myers *et al.*, 2007). In 1988, a woman contracted variant IAV and subsequently died after attending a county fair where ill swine had been reported (Wells *et al.*, 1991). In 2007, genetically identical H1N1 isolates were recovered from humans and swine at a county fair in Ohio, suggested direct transmission of IAV from the pigs to the humans occurred at the fair (Killian *et al.*, 2013). Sub-clinically infected swine can contribute to zoonotic IAV transmission, as occurred in 2009 when a 12 year old boy contracted variant H3N2 IAV after petting apparently healthy swine at a county fair in Kansas (Cox *et al.*, 2011). In 2011, multiple cases of variant IAV were associated with exposure to swine at agricultural fairs in Pennsylvania (Wong *et al.*, 2012). The number of variant IAV cases in humans spiked in 2012, with 306 cases of variant H3N2 IAV (Jhung *et al.*, 2013). With the advent of sequencing technology many of these cases can be directly classified as swine lineage IAV through molecular epidemiology and linked to swine exposure at agricultural fairs (Bowman, 2014). These cases illustrate that infection with variant IAV can result in human hospitalizations and in some cases death.

Surveillance conducted from 2009 to 2011 identified one or more IAV-infected pig(s) at the end of approximately one quarter of the agricultural fairs tested in Ohio (Bowman, 2012). Of the fairs with infected swine, the average frequency of virus isolation was 62.9% (Bowman, 2012) indicating that a high proportion of the pigs at
those exhibitions were actively shedding IAV at the end of the 5-7 day fair. However, little is known about IAV prevalence in swine arriving at exhibitions before swine have commingled and IAV spread through the population. Previously, one study found a large variation in incoming prevalence estimates across two study sites, ranging from 0% to 19% via rRT-PCR testing (Gray, 2012). However, only a small proportion of the swine population were tested. A study by Bowman et al. found the prevalence of IAV at one state fair to be 2.4% among the incoming swine (Bowman, In Press). The objective of the present study was to estimate the prevalence of IAV in exhibition swine as they enter fairs as a prelude to investigating the prevalence of IAV following commingling at the exhibition site.

2.4 Materials and Methods

Enrollment of fairs

Nine agricultural fairs (labeled A through I) that previously enrolled in an Ohio State University IAV surveillance program were recruited for the present study. Exhibitions were selected based on willingness to participate in the program, previous history of IAV in their exhibition swine, the number of swine that were historically exhibited at the fair, and the date of exhibition that allowed for proper sampling. Due to the need for multiple sample collectors in the field during the fair season and a self-imposed three-day downtime between fairs for investigators to minimize risk of transmitting IAV between exhibitions, fairs were selected to minimize overlap in sampling dates. The nine fairs sampled occurred in July and August of 2014, with four fairs sampled in Ohio and five sampled in Indiana.
**Sampling of swine**

All swine sampled at the exhibitions were sampled at the first time point that they could be individually identified. This sampling occurred on the trailer before unloading (Exhibition A), in the pen prior to weighing (Exhibitions D and E) or in a chute as swine were moved individually through a narrow series of gates and weighed on a scale (Exhibitions B, C, F, G, H, and I). Study team members targeted all swine entering Exhibitions B through I for sample collection. At Exhibition A, sample size was intentionally restricted in an effort to expedite sampling because swine were sampled on the trailers during arrival to the fair. For each participating trailer at this exhibition, study team members were instructed to sample no more than 2 swine that were easily accessible without entering the trailer. Sampling at all exhibitions was performed via the snout wipe method as previously described (Edwards, 2014). Vials were stored at -70°C until testing was completed. The Ohio State University Institutional Animal Care and Use Committee approved sampling of animals in this study (protocol no. 2009A0134-R1)

**Laboratory processing**

Original samples were quickly thawed and RNA was extracted using a laboratory modified protocol for a 100µL sample extraction using the Mag-Bind® Viral DNA/RNA 96 Kit¹ and a MagMAX™ Express 96 Magnetic Particle Processor² (AM1836_DW_100_V2 program). The modified protocol used 120µL TNA lysis buffer, 140µL isopropanol, 4µL Carrier RNA, 2µL internal positive control template, 7µL proteinase K per reaction well. Additionally there were two washes with 200µL VHB buffer and two washes with 200µL SPR buffer. RNA was eluted into 50µL nuclease-free

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¹ Omega Bio-tec Inc.®, Norcross, GA, USA
² Applied Biosystems®, Foster City, CA, USA
water. Sample RNA was screened via a one-step real-time reverse transcription-polymerase chain reaction (rRT-PCR) for IAV\(^3\). Original samples were once again frozen at -70°C while rRT-PCR was being performed and analyzed. Any samples demonstrating a cycle threshold (C\(_t\)) value \(\leq 35\) was considered rRT-PCR positive for IAV. Positive samples were rethawed and each treated with 120μg amphotericin, 5.000mg gentamicin sulfate and 1.625mg kanamycin sulfate. Samples were vortexed and inoculated into 4 wells of a 24 well plate with monolayers of serum-free-adapted Madin-Darby canine kidney (MDCK) cells (Bowman \textit{et al.}, 2013). Cells were observed daily for 72 hours post inoculation for cytopathic effects (CPE). Upon harvest, cell culture supernatant was tested for hemagglutinating activity using 0.5% turkey erythrocytes (Hierholzer \textit{et al.}, 1969). Samples demonstrating CPE and/or hemagglutination were tested via a rapid strip test for the p56 nucleoprotein of IAV\(^4\). If the sample had an initial C\(_t\) value \(\leq 30\) and IAV was not isolated during the first passage in MDCK cells, a second passage was attempted (Zhang, 2014c). Recovered isolates were subtyped with a multiplex hemagglutinin and neuraminidase rRT-PCR assay\(^5\). Matrix gene lineage was determined to be either the North American swine triple reassortant lineage or influenza A(H1N1)pdm09 virus lineage through a multiplex rRT-PCR (Harmon \textit{et al.}, 2010). Viral isolation data was used to determine the incoming prevalence of IAV since rRT-PCR does not differentiate between residual RNA and active virus, whereas virus isolation demonstrates that infectious IAV was recovered from the snout of the pig during sampling.

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\(^3\) VetMAX-Gold SIV Detection Kit; LifeTechnologies®, Austin, TX, USA  
\(^4\) FluDETECT® Avian Influenza Virus Type A Antigen Test Kit, Synbiotics Corporation, San Diego, CA, USA  
\(^5\) Life Technologies®, Carlsbad, CA, USA
2.5 Results

A total of 3,547 samples were collected from the 5,462 swine in attendance at the nine exhibitions. Of the samples collected, 188 (5.3%) were IAV positive using rRT-PCR and viable IAV was recovered from 53 (1.5%) (Table 2.1). Within exhibitions A through E, IAV prevalence, as determined by virus isolation, ranged from 0.2% to 10.3%. No IAV was detected in the samples collected from the swine at Exhibitions F, G, H, or I. Overall, IAV isolates were recovered from 28.2% of the samples identified as rRT-PCR positive but there was a wide range in isolation success from rRT-PCR positive samples between fairs. For Exhibition E, 100% of the rRT-PCR positive samples yielded an IAV isolate, whereas at Exhibition C only one IAV isolate was recovered from the 16 rRT-PCR positive samples (6.2%). Forty-seven (88.3%) of the 53 isolates were recovered during the first passage and the remaining 6 isolates were recovered through a second passage.

The 53 isolates were subtyped as H1N1 (n=23), H3N2 (n=28) and mixed with both H1/H3 N1/N2 subtypes (n=2) (Table 2.2). All IAV isolates contained the influenza A(H1N1)pdm09 lineage matrix gene. In Exhibition A, both H1N1 (n=2) and H3N2 (n=4) subtypes were recovered. Similarly, H1N1 (n=18), H3N2 (n=23) and mixed subtype isolates (n=2) were found at Exhibition B. Only one IAV subtype per exhibition was detected among the swine entering exhibitions C-E (Table 2.2).

At the two fairs where swine were sampled in a chute and swine tested positive for IAV (Exhibitions B and C), a pattern in rRT-PCR values for IAV was observed at both fairs. As illustrated in Figure 2.1 (Panel A and B), samples with a low rRT-PCR Ct value were often followed by samples with similarly low Ct values that would gradually
increase over subsequent samples (time) until the next low C\textsubscript{t} spike. This pattern was not observed at Exhibitions D and E, fairs where IAV positive swine were detected but the swine were not sampled in a chute (Figure 2.1, Panel C).

2.6 Discussion

With viable IAV being recovered from only 1.5% of the swine entering the sampled fairs, findings of the present study highlight the potential to, and importance of, limiting the intra-species spread of IAV during swine exhibitions. Since swine are cohoused for several days during an exhibition, viruses arriving at the beginning of the fair have ample time to spread through the swine population. Surveillance for IAV in swine conducted at the end of exhibitions indicates that when IAV is among the swine at a given fair, viable IAV is typically recovered from 60-70% of the pigs (Bowman, 2012, Bowman, 2014). This demonstrates a remarkable increase in IAV prevalence among swine between entry to the exhibitions and end of exhibition. Certainly the risk to public health in these settings increases as the IAV prevalence among the exhibition swine grows during the fair, a notion supported by the timing of variant IAV detection in relation to the implicated fair (i.e. when variant cases detected, they are almost always detected at the end of the fair or in the days immediately following conclusion of the fair). Strategies to maintain a very low prevalence of IAV within an exhibition site, thus preventing the apparent amplification of IAV during fairs, have the potential to reduce the threat to public and swine health. One such strategy is shortening the swine exhibition period (Bowman, 2014, NASAHO, 2014).

Subclinical infections of IAV occur in pigs and have been detected at 83.3% of agricultural fairs with IAV infected swine (Bowman, 2012). This provides the additional
challenge that IAV infected swine cannot be identified by clinical signs during entry. Attempts to use infrared and rectal thermometers have also been unsuccessful for screening pigs of IAV (Bowman, In Press). Ultimately, the IAV status of the swine can only be determined by diagnostic testing. Given the extensive spread of IAV within the population of swine at an exhibition, a viable method to detect the small percentage of IAV positive pigs at entry and prevent their entry to the exhibition site would be the ideal outcome. However, this option is currently an unrealistic proposition since the labor, cost, and time required to perform diagnostic testing would be beyond the capacities of most exhibitions. Mitigation strategies to avert swine-swine transmission of IAV during the fair will likely decrease the total IAV burden in swine barns at fairs and reduce the risk of zoonotic IAV transmission.

For the present study, the ideal time point for sampling was on trailer prior to unloading, as was performed at Exhibition A, since the swine were not yet exposed to the exhibition’s animals or environment. Given logistic complications, this approach was not feasible at the other exhibitions. The maximum time that swine were at Exhibitions B through I prior to sampling was 24 hours. Swine at Exhibitions D and E were sampled in their pens after unloading but prior to weighing. The swine at the remaining exhibitions were sampled in the chute during weighing. The clustering at Exhibitions B and C observed in Figure 2.1 (Panel A and B) are similar, however the amount of IAV recovered differed greatly between the two exhibitions. At Exhibition B, 34.8% of the samples were rRT-PCR positive for IAV; and 43 IAV isolates were recovered. In the case of Exhibition C, 4.5% of the samples were rRT-PCR positive for IAV; and one isolate was recovered.
Temporal clustering of positive swine within fairs was expected because swine from the same farm: were predicted to have similar IAV exposure, would arrive at the exhibition together, be placed in related pens, and moved together through the chute. However, the large proportion of samples testing IAV positive with rRT-PCR and the unusual trailing off of Ct values observed at Exhibitions B and C (Figures 2.1: Panel A and B) was not anticipated. The most likely explanation for this trend is swine snout contamination during corralling activities. In this scenario, an IAV infected pig deposits virus via oral and/or nasal secretions onto swine contact surfaces as it moves through the chute. Subsequent swine moving through the chute have the opportunity to contact the contaminated surface(s) and acquire IAV on their snouts. IAV concentration on the surfaces, and thus the snouts, would be expected to diminish over time as multiple swine remove IAV from the contaminated gating during transit through the chute. Although genomic sequencing is needed to fully assess IAV isolate identities, the temporal clustering of identically subtyped isolates, as observed at Exhibition B (Figure 2.1: Panel A), provides additional support for this hypothesis. One would expect a more dispersed distribution of subtypes across the sampling if the 43 IAV positive swine at Exhibition B were in fact infected prior to arrival at the exhibition. We hypothesize that moving swine through a chute has the potential to expedite pathogen transmission during the exhibition because this practice allows many swine to be rapidly exposed to a variety of pathogens within hours of arrival. This deposition of IAV onto the snouts of swine during corralling likely results in faster IAV transmission than if the virus had to spread pig-to-pig via direct contact. IAV can be transmitted between swine via fomites (Allerson et al., 2013), thus it is possible that common contact surfaces within the chute (i.e. gates, handing
equipment, walls, scale, etc.) could facilitate IAV spread. Corralling activities of swine, and similar situations during the exhibition, may be critical points for limiting IAV transmission at fairs. Future work is needed to assess IAV contamination of chutes and similar surfaces at fairs. Attention to reducing contamination of these surfaces (e.g. frequent disinfection) may be warranted.

Other studies investigating IAV prevalence among incoming exhibition swine found discrepancies similar to what was observed between fairs in the present study. During 2008-2009 Gray et al. conducted a surveillance study at fairs in Minnesota and South Dakota. No IAV was detected in the swine sampled at the Minnesota fair in 2008; however in 2009, 19.3% of the swine at the same Minnesota fair and 2.2% of the swine at a South Dakota fair were PCR positive for IAV (Gray, 2012). This study provided an insightful preliminary look at IAV among swine at exhibitions; however, the sample size was relatively small and timing of sample collection relative to arrival was loosely defined making on-site IAV transmission prior to sampling possible. In a 2013 pilot study Bowman et al., recovered IAV isolates from 2.4% of samples collected from swine on trailers prior to unloading at Exhibition A (Bowman, In Press).

There are some limitations with the prevalence established during the present study. It was necessary, due to time and funding restraints, to screen samples with rRT-PCR before inoculating them for virus isolation. Therefore, all samples were subject to a freeze-thaw cycle prior to a virus recovery attempt. Freeze-thaw cycles have been associated with decreased IAV infectivity and thus, the frequency of virus isolation in the present study is likely lower than if samples had been directly inoculated without refreezing during rRT-PCR screening (Greiff et al., 1954). Additionally, the snout wipe
method used in the current study is less sensitive than the gold-standard nasal swabs (Edwards, 2014). Snout wipes were chosen, as they are noninvasive, do not require restraint, and take less time to administer than nasal swabs. In the present study, exhibitors were very concerned about stress on their animal, even momentary stress, therefore, snout wipes were chosen to maintain a level of acceptance from the exhibitor to the sampling process. It is important to also note that snout wipes are taken from the surface of the pig’s snout and may represent more of a conglomerated sample of the pig and its environment when compared with a gold standard nasal swab that requires pig restraint. The possible cross-contamination was observed at Exhibitions B and C, creates the potential that the individual animal IAV prevalence was overestimated in the present study. Based on the IAV subtypes identified at Exhibition C, we suspect at least 3 swine had active IAV infections during the time of sampling at Exhibition C.

In conclusion, the current project demonstrated that a small number of swine arrived at exhibition actively infected with IAV. However, the estimated prevalence of IAV at individual fairs ranged widely and may in-part be due to the slight difference in sampling procedures used at each fair. In particular, sampling that occurred in chutes resulted in a far greater prevalence of IAV when compared with sampling in pen or on a trailer. We hypothesize this to be the result of IAV contamination in the chute. If true, fomite transmission of IAV may occur whenever swine are moved through the barn such as during weighing, washing, walking to and from the show ring, and at times when animals encounter contaminated sites. These activities could be heightened areas for IAV transmission between swine, and thus, potential time points and locations to target for controlling swine-to-human zoonosis. Prevention strategies targeted at limiting IAV
spread during the fair should be developed to limit the risk of IAV infections in humans and swine at agricultural exhibitions.

2.6 Acknowledgements
We thank our collaborators from the participating agricultural fairs. We also thank Alexa Edmundson, Elise Gerken, Amber Kihm, Grant Price, Christine Szablewski, and Jeffrey Workman for their assistance in sample collection. This work was supported in part by National Pork Checkoff; and with federal funds from the Centers for Disease Control and Prevention, Department of Health and Human Services, under Cooperative Agreement U38OT000143 and from the Centers of Excellence for Influenza Research and Surveillance (CEIRS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract number and HHSN272201400006C.
### Table 2.1: Sampling results for influenza A virus in swine at the beginning of nine agricultural fairs, 2014

The nine agriculture exhibitions where snout wipes were collected from swine in 2014 are displayed. The sampling location column indicates if the snout wipes were collected on the trailer prior to unloading, in the pig’s pen prior to weighing, or in the chute during weighing. No. swine tested shows the number of samples per fair that were screened for influenza A virus (IAV) via rRT-PCR. The number of samples that were rRT-PCR positive ($C_t \leq 35$) for IAV and the number of IAV isolates recovered from cell culture are listed.

<table>
<thead>
<tr>
<th>Fair</th>
<th>Sampling location</th>
<th>No. of swine exhibited at fair</th>
<th>No. (%) swine tested</th>
<th>No. (%) rRT-PCR positive</th>
<th>No. (%) virus isolation positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhibition A</td>
<td>Trailer</td>
<td>2149</td>
<td>382 (17.8%)</td>
<td>21 (5.5%)</td>
<td>6 (1.6%)</td>
</tr>
<tr>
<td>Exhibition B</td>
<td>Chute</td>
<td>424</td>
<td>419 (98.8%)</td>
<td>144 (34.4%)</td>
<td>43 (10.3%)</td>
</tr>
<tr>
<td>Exhibition C</td>
<td>Chute</td>
<td>377</td>
<td>359 (95.2%)</td>
<td>16 (4.4%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Exhibition D</td>
<td>Pen</td>
<td>465</td>
<td>445 (95.7%)</td>
<td>6 (1.4%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>Exhibition E</td>
<td>Pen</td>
<td>523</td>
<td>523 (100.0%)</td>
<td>1 (0.2%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Exhibition F</td>
<td>Chute</td>
<td>367</td>
<td>367 (100.0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition G</td>
<td>Chute</td>
<td>274</td>
<td>274 (100.0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition H</td>
<td>Chute</td>
<td>597</td>
<td>492 (82.4%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition I</td>
<td>Chute</td>
<td>286</td>
<td>286 (100.0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>5462</strong></td>
<td><strong>3547 (64.9%)</strong></td>
<td><strong>188 (5.3%)</strong></td>
<td><strong>53 (1.5%)</strong></td>
</tr>
</tbody>
</table>
Table 2.2: Influenza A virus subtypes recovered from incoming swine at agricultural fairs, 2014. Surveillance for influenza A virus at nine agricultural fairs in 2014 was conducted on swine during their arrival to the exhibition. The five agricultural exhibitions where influenza A virus isolates were recovered via cell culture are displayed by their hemagglutinin and neuraminidase subtype.

<table>
<thead>
<tr>
<th>Fair</th>
<th>No. (%) H1N1 IAV</th>
<th>No. (%) H3N2 IAV</th>
<th>No. (%) mixed subtype IAV, H1/H3 and N1/N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhibition A</td>
<td>2 (33.33%)</td>
<td>4 (66.67%)</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition B</td>
<td>18 (41.86%)</td>
<td>23 (53.50%)</td>
<td>2 (4.65%)</td>
</tr>
<tr>
<td>Exhibition C</td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition D</td>
<td>2 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exhibition E</td>
<td>-</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23 (43.40%)</strong></td>
<td><strong>28 (52.83%)</strong></td>
<td><strong>2 (3.77%)</strong></td>
</tr>
</tbody>
</table>
Figure 2.1: Prevalence of influenza A virus among swine entering Exhibitions.

The horizontal axis represents the relative order of individual swine as they were sampled. In Panel A (Exhibition B) and Panel B (Exhibition C) swine were sampled as they moved through a narrow passage and a series of gates, collectively known as a “chute”, for arrival process. Panel C (Exhibition E) swine were sampled in pen prior to being moved through a chute. The snout wipe sample collected was screened for influenza A virus via rRT-PCR. The Ct value determined for each individual pig is displayed on the vertical axis. The black line at 35 indicates the cut point for a positive rRT-PCR sample. Closed black circles indicate recovery that an isolate with H1N1 subtype, open circles indicate recovery an isolate with H3N2 subtype and black stars indicate a mixed isolated with H1/H3 and N1/N2 subtypes. Note that there appears to be a temporal relationship between the order of the individual pig in the samples and the Ct value displayed in the collected sample.
CHAPTER 3
On-farm management practices of exhibition swine and associations with influenza A virus infections

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Objective- To identify the geographic distribution of exhibition swine at the county fair level in the Midwestern United States, characterize the management practices used on exhibition swine farms, and identify associations between practices and influenza A virus prevalence in exhibition swine arriving at agricultural fairs.

Design- Cross-sectional surveys.

Animals - 641 exhibition swine were tested for influenza A virus and linked with surveys administered to swine exhibition participants
Procedure – By county in 2013, fair organizers and state animal health officials reported total number of exhibition swine attending fairs. In 2014, Snout wipes were collected from exhibition swine during their arrival to fairs and linked to a 24-question written survey concerning on-farm management practices was administered to exhibition participants. Snout wipes were screened for influenza A virus using rRT-PCR.

Results- The exhibition swine population in the Midwestern United States appears to be more heavily concentrated in Indiana and Ohio, whereas Midwestern commercial swine populations are heavily concentrated in Iowa. Participants returned 480 surveys which were linked directly with 641 exhibition swine. Respondents reported a high level of swine movement, with multiple exhibition attended (mean 3, median 2, range 0-50), often returning to their originating premise. Both commercial swine production and raising of exhibition swine was reported to occur at the same premises by 13.3% of the participants. The management practice of hosting an open house/sale on-farm was found to be significantly associated to the detection of IAV in pigs arriving to the fairs (OR = 3.93; 1.10-13.06).

Conclusions and Clinical Relevance – The exhibition swine population differs from the commercial swine population in geographical location, population integrity and on-farm management practices. On-farm management practices of exhibition swine are highly variable. As exhibition swine may serve as an important reservoir for influenza A viruses, identified biosecurity deficiencies may have important public and agricultural health consequences.
3.1 Introduction
Recently, zoonotic transmission of swine-lineage IAV to humans, termed a variant IAV, has gained public health attention (WHO, 2014). Since 2005, there have been 373 reported human cases of variant IAV in the United States (CDC, 2014d), with many additional infections likely unreported (Biggerstaff et al., 2013). The individuals most likely to suffer severe consequences from variant IAV infections are people in high risk groups, such as children under five years of age, people over sixty-five years of age, pregnant women, and immunocompromised individuals (Gambhir et al., 2013). Zoonotic transmission events of IAV between swine and humans have been most frequently documented at the human-swine interface at agricultural fairs (Bowman, 2014, Jhung et al., 2013, Myers et al., 2007).

Exhibition swine are recognized to be unique in the swine industry, as they may be shown multiple times across locations in exhibitions that are open to any age competitor. Though the exhibition swine industry accounts for an estimated 1.5% of the total swine population in the United States (National Swine Registry, personal communication, 2015), these swine come into contact with the general public and swine from multiple locations at agricultural fairs making them a potentially important reservoir for IAV. Thunes et al. (2007) reported that 97% of exhibitors returned to their farm from the California state fair with their animals. Wayne et al. (2012) provided the first look at exhibition swine management, highlighting that 39% of the Minnesota state fair participants raised commercial and exhibition swine at the same location. This practice could promote IAV transmission in the swine industry facilitating the movement of pathogens between these two swine populations.
The National Association of State Public Health Veterinarians (NASPHV), National Assembly of State Animal Health Officials (NASAHO), Centers for Disease Control and Prevention (CDC) and National 4-H Headquarters have put forth a number of mitigation strategies and suggestions for controlling the movement of IAV in exhibition swine populations and decreasing human exposure to IAV (NASAHO, 2014). On the human side, people at high risk of serious IAV complications are advised to avoid contact with swine or the environment of swine. Those individuals not at high risk, are generally told to avoid or minimize contact with ill swine, practice good hand hygiene, and use personal protection if coming into direct contact with ill swine. The CDC also recommends that swine caretakers receive seasonal influenza vaccinations (CDC, 2014a). Swine owners are encouraged to vaccinate swine for IAV and to contact a veterinarian if swine are noticed to have ILI (CDC 2013, NASAHO, 2014). Other suggestions include the implementation of biosecurity measures, such as to isolate and observe swine for ILI for seven days after exhibition attendance, clean and disinfect equipment, and maintain adequate ventilation within the swine barns (CDC, 2014b, NASAHO, 2014). While these general measures are expected to decrease the likelihood of swine-human IAV transmission, more specific recommendations to control IAV spread have not been made because there is a paucity of data about the unique exhibition swine population and the animal management practices used within this niche industry. Previous to the current study, the geographical location of the exhibition swine population was unknown relative to the total swine industry. Reports of variant IAV do not correlate geographically with the larger commercial swine populations in Iowa, North Carolina, and Minnesota. Out of the sixteen states that have reported variant IAV since 2005, over 70% of these cases
have occurred in Indiana (n = 154) and Ohio (n = 112) (CDC, 2014d); states that ranked 5\textsuperscript{th} and 10\textsuperscript{th} respectively in total swine inventory (USDA, 2012).

The current study sought to identify the geographic distribution of the exhibition swine industry in the Midwestern United States, describe on-farm management practices used in the exhibition swine industry, and identify associations between practices with IAV detection in exhibition swine arriving at agricultural fairs.

### 3.2 Material and Methods

**Geographic distribution of exhibition swine**

State animal health officials, county Extension educators, and/or local fair organizers in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio were contacted to determine the number of swine exhibitions and swine exhibited at county/local agricultural fairs during 2013. State fairs and other swine exhibitions were excluded. The total swine population data for each county was obtained from the 2012 United States Agricultural Census (USDA, 2012). The reported swine number and the number of exhibition swine per county were interpolated to the geometric centroid of each county. A continuous spatial distribution for each population was developed using inverse distance weighting based on 15 neighbors\(^6\). An eight tier geometric geometrical interval color scale was used to generate a visual heatmap.

**Survey administration**

A twenty four question paper survey containing close-ended questions was administered in English to the adults accompanying the exhibition swine at nine agriculture fairs across Ohio and Indiana during July and August 2014\(^7\) (Appendix A).

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\(^6\) ESRI ArcMap 9.3.1.

\(^7\) Available upon request from corresponding author.
Surveys were modeled after a previous survey administered fair officials (Bowman et al., 2014). No personal identifying information was collected but participants were asked to provide the individual identification number of their swine, allowing survey and pig data to be linked. The study team did not have a list of exhibitors and therefore could not trace the results back to a specific person or farm. Participants were asked to only complete one survey per exhibition swine rearing premise (i.e. one survey per household that might represent multiple exhibition swine). Due to the differences in the individual management practice of the fairs, surveys were administered in two ways. At Exhibition A, surveys were administered in-person while swine were being sampled and made available to all income participants. At Exhibitions B-I, surveys were distributed to participants at the beginning of the fair and collected via a centrally located drop box throughout the exhibition. The research plan was exempted from review by The Ohio State University Institutional Review Board under protocol no. 2014E0141.

**Sampling for IAV at fairs**

Snout wipes were collect from exhibition swine arriving at agricultural fairs A-I as part of a previously described prevalence study (Chapter 2). Samples were tested for IAV using rRT-PCR and samples with an rRT-PCR cycle threshold values ≤ 35 were classified as IAV positive (Chapter 2). The IAV test results of the incoming swine were then linked to the corresponding survey. The Ohio State University Institutional Animal Care and Use Committee approved under protocol no. 2009A0134-R1.

**Statistical analysis**

Survey responses were entered into a statistical program, screened and obviously spurious results (such as housing swine with aquatic mammals) were dropped from the analyses. Descriptive statistics were calculated for all variables. Surveys that were not
associated with individual swine (i.e. no swine identification was provided) were used for descriptive analyses of on-farm management practices but were dropped from the IAV risk factor analyses. Univariate exact logistic regression was used to individually examine reported on-farm management practices to identify risk factors associated with IAV positive swine arriving at exhibitions\(^8\). A \(P\)-value < 0.05 was considered significant.

### 3.3 Results

Data for county exhibition swine were received from 553 of the 578 (95.7\%) counties in the six states. The average number of swine exhibited per county fair ranged from 53.5 in Missouri to 226.8 in Indiana (Table 3.1). Mapping these data illustrated the exhibition industry to be concentrated in Indiana and Ohio (Figure 3.1), whereas the total swine population was concentrated in Iowa (Figure 3.2).

Surveys were collected from all nine fairs and fair organizers reported 3662 swine exhibitors in attendance. A total of 480 surveys were collected of which, 52 were dropped from the study due to illegibility or identifiable issues with answers provided. The remaining 428 (89.2\%) were used for analyses. Descriptive statistics of the continuous response variables surrounding swine management are displayed in Table 3.2 and binary variables are displayed in Table 3.3. The median number of swine on participants’ farms on January 1\(^{st}\) 2014 was zero, reflecting that swine were not housed year round on half of the premises. Participants reported showing swine at an average of 3.4 exhibitions prior to arrival at the county fair under study (median, 2; range, 0-50). Respondents indicated that they returned from prior exhibitions with swine an average of 2.9 exhibitions (median, 2; range 0-40). Swine were obtained from an off-farm source by

\(^8\) STATA version 11.1, StataCorp, College Station, TX, USA.
75.4% of the survey participants, with 84.3% of the purchases occurring between March and April. Exhibition swine were raised in small herds (maximum no. of swine on farm; median, 6) and typically housed in an open building with natural ventilation and no outside access (47.5%) or an open building with outside access (39.2%). Of those who returned home from an exhibition with their swine, 48.6% implemented some form of isolation for returning swine. Additionally, 45.4% of the respondents reported that exhibitors and/or household members had contact with swine or the environment of swine, other than their own, at least weekly. Commercial swine production was reported for 13.3% of the same premises as where the exhibition swine were raised.

Vaccination against IAV was reported for 62.7% (n=197) of the swine entering the nine exhibitions (Table 3.4). Of the vaccinated swine 7.8% were rRT-PCR positive for IAV and IAV isolates were recovered from 2.6%. The income prevalence among non-vaccinated swine was 2.9% via rRT-PCR and IAV isolates were recovered from 1.0%.

Hosting an open house/sale on-farm was the only management practice significantly associated with prevalence of IAV at arrival to fairs (OR=3.93; CI: 1.10-13.06).

3.4 Discussion
The present study identified that exhibition swine differ in geographic concentration, population integrity, and on-farm management practices relative to the commercial swine industry. Though still poorly defined, an interaction between the commercial and exhibition swine populations was identified occur on the premises of the participants.
The density maps constructed from the participating states illustrate that the exhibition swine are concentrated in different locations than the commercial swine population. The commercial swine population was represented by the total swine population reported in the 2012 USDA census and thus is reflective of current inventory of swine on-farm in 2012. Recalling that exhibition swine account for an estimated 1.5% of the swine population, total swine inventory represents an estimate of commercial swine location by county. For privacy, county data may have been blinded if individual operations can be identified in the 2012 USDA census (i.e. only one producer in the county) posing a potential bias for the density maps. A previous study suggested that the occurrence of variant IAV cases were linked to commercial swine density (Jhung et al., 2013), however visual examination of the data suggests the locations of the variant IAV cases are more closely allied with the exhibition swine population. Swine attending the state fairs were not displayed in the density map due to our desire to characterize where exhibition swine are raised geographically. Typically swine attending county fairs are raised in closer proximity to the fair, than swine attending state fairs. Additionally, we did not want to count individual pigs twice if they attended both the county and state fairs. Our study shows the majority of exhibition swine among participating states were concentrated in Indiana and Ohio, where over 70% of the United States variant IAV cases have been reported, suggesting exhibition swine density may play a role in the incidence of variant IAV. It is logical that areas with larger exhibition swine populations would have a larger human-swine interface at which transmission of this pathogen can occur. Bowman et al. (2014) has shown that the odds of having IAV infected swine at fairs were
1.27 (95% CI: 1.04-1.66) higher for every 20 swine increase in the size of the swine show.

Our study shows that the exhibition swine industry raises swine on small farms that utilize an assortment of swine management practices. Nearly half of the participants reported having direct contact with other’s swine (or their environment) at least weekly, demonstrating that these caretakers commonly move between different groups of swine, a practice that is strongly discouraged and often disallowed in commercial swine production.

Since the majority of exhibition swine are exhibited in the Midwestern USA are obtained in late spring and the present study sampling occurred in mid-summer, the participants had only a three to four month time period to show their swine and attend the relatively large number of exhibitions reported. With the successful elimination of pseudorabies virus from United States swine herd (USDA, 2008), the legislative mandate of terminal swine shows has been lifted and non-terminal shows have become common. Exhibition-to-exhibition movement of swine creates a pathway for the rapid dissemination of many pathogens, including IAV. The detection of highly identical IAV strains among exhibition swine across Ohio during 2012 was likely the result of this inter-exhibition swine movement (Bowman, 2014). Nelson et al. (2015) identified that exhibition swine in Ohio and Indiana frequently shared related IAV strains indicating frequent viral movement within this swine-dense exhibition region. Additionally, the return of swine to farms after exhibition creates a threat for future exhibitions because IAV introduced to naïve swine on-farm will further perpetuate IAV transmission, which newly infected swine on the farm may carry with them to future exhibitions. Thus,
limiting swine movement and performing the suggested 7 day on-farm isolation of returning swine is vital for the control of IAV between exhibitions. If swine display ILI during their isolated time, they should remain on farm until IAV infection has fully cleared. While almost half of the participants in the present study already implemented some form of isolation for returning swine, there was a wide range in the reported isolation period (2 hours to 30 days), highlighting the need for better education of exhibitors about effective biosecurity measures.

With 62.7% of the reported swine having received an IAV vaccination, it appears that exhibitors are following the suggested measure of vaccination put forth by officials, however having a vaccinated status for IAV was not correlated with a decreased of IAV in vaccinated swine. Work done by Loving, et al. has shown that IAV vaccination can eliminate clinical signs of disease in swine, but is not completely effective at blocking infection and pathogen transmission (Loving et al., 2013). Thus, in these exhibition swine populations the subclinical infections that have been report in Ohio (Bowman et al., 2014) and Minnesota (Gray et al., 2012) may be due to suppression of the clinical signs by vaccination.

The high level of comingling and movement occurring in the exhibition swine industry is distinctly different than what occurs in commercial swine production. Commercial swine are raised together in large herd sizes, averaging 1,044 head per farm (USDA, 2012). Commercial swine herds typically maintain a high degree of population integrity, remaining essentially stable with few, if any, swine entering, exiting, and reentering. Utilizing all-in-all-out management, commercial swine are typically moved two to three times during their life, corresponding with movement to a new location at
weaning (to a nursery or directly to a finisher), and or removal from the nursery phase directly to the finisher phase, and transfer from the finisher directly to the end market destination.

While both commercial and exhibition swine have contact with humans, the demographic of the human population that exhibition swine can contact at agricultural fairs likely have little to no previous exposure to swine. In particular, at county fairs there are a large number of youth (8-18 years of age) exhibiting swine. During the fair, these children are in prolonged, close contact with exhibition swine. The findings by Skowronski et al. illustrate that children represent a naïve population to swine lineage IAV, as they been found to have little to no existing antibodies present to common variant subtypes (Skowronski et al., 2012).

Interestingly, 13.3% of survey participants reported commercial swine production on the same premise as exhibition swine. This is lower than the previously reported 39% that had been found in Minnesota (Wayne et al., 2012). This difference may be reflective of the large exhibition swine population in Indiana and Ohio and the relatively smaller commercial swine populations, or be indicative of the larger population base from which youth exhibitors are drawn, leading to added interest and opportunity for youth from non-farm backgrounds to raise livestock on a temporary basis, on a small scale, rural setting. Surprisingly, swine are returning from exhibitions to locations where commercial swine production is reported, a clear violation of common disease prevention biosecurity practices. Though interaction between the two swine populations on-farm is not known (i.e. are the commercial and exhibition swine housed together or separate), movement of swine onto a farm is a known risk factor for IAV infections (Poljak et al., 2008). While
the magnitude of interaction is still poorly defined, the interface between commercial and exhibition swine described in this study may facilitate the introduction of IAV strains generated at exhibitions into the commercial swine population as described by Nelson, *et al.* (2015).

The present study also identified that twenty one premises (37.5%) with commercial swine production and forty premises (11.0%) without commercial swine production reported hosting an open house/sale on-farm where visitors were welcomed on to the farm. Though hosting an open house/sale on the premise with exhibition swine was found to have a significant relationship with the prevalence of IAV among swine eventually arriving at a county fair sampled, we believe this to be a representation for professional swine exhibitors. Professional swine exhibitors, people who breed, raise and exhibit swine for a living, likely host open houses/sales to sell their swine. These exhibition swine producers would also be expected to attend a larger number of exhibitions during the year, providing their swine with additional opportunities for IAV exposure. In the current study it was found that those who hosted open house/sales reported attending significantly more exhibitions (mean number of exhibitions 5.45, \( p = 0.017 \)) then those who did not (mean number of exhibitions = 2.67).

The current study contained several limitations, one of which was the self-reported nature of the survey data. Participants classified themselves as raising commercial swine without any guidance from the study team. In addition, participants did not receive assistance or guidance from the study team; therefore, misunderstanding may have occurred relative to some questions and the subsequent response. Additionally
response bias, recall bias and misclassification are possible biases for this study. It should
be noted that the 52 surveys that were dropped from this study were mostly from
incompletion of questions, or response that brought the integrity of the data into question.

While exhibition swine represent only a small percentage of the total swine population, they are often considered the face of the swine industry to the public.
Exhibitions allow a physical interface with general public, an interface with exceptional
tportunity to present and showcase agriculture, but also an interface with unique
challenges in disease control across the swine industry and the potential for zoonosis, a
cern for public health. Results of the present study identify unique management
areas within the exhibition swine industry, areas that pose risk and opportunity in relation
to the general public. The findings support development of biosecurity tools and
education as mitigation strategies to prevent IAV transmission in exhibition swine and
across the human-animal interface.
3.5 Acknowledgements

We thank our collaborators from the participating agricultural fairs. We also thank Jody Edwards, Alexa Edmunson, Elise Gerken, Amber Kihm, Sarah Nelson, Jacqueline Nolting, Grant Price, Christine Szablewski, Jeffrey D. Workman and Michele Zentkovich for their assistance and support in this study. This work was supported in part by National Pork Checkoff; and with federal funds from the Centers for Disease Control and Prevention, Department of Health and Human Services, under Cooperative Agreement U38OT000143 and from the Centers of Excellence for Influenza Research and Surveillance (CEIRS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract number and HHSN272201400006C.
Tables and Figures

**Figure 3.1:** The reported geographical distribution of exhibition and commercial swine in the Midwestern United States (2013). Panel A: The number of exhibition swine at county fairs during 2013 in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio displayed in a heat-map. Dark red indicates the highest density of swine and dark blue represents the lowest density of swine. Panel B: Total swine inventory for each county in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio from the USDA 2012 Census of Agriculture, displayed in a heat-map. Dark red indicates the highest density of swine and dark blue represents the lowest density of swine.
<table>
<thead>
<tr>
<th>State</th>
<th>Average number of swine per county</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>65.3</td>
<td>71.6</td>
</tr>
<tr>
<td>Indiana</td>
<td>226.8</td>
<td>132.0</td>
</tr>
<tr>
<td>Iowa</td>
<td>123.0</td>
<td>76.5</td>
</tr>
<tr>
<td>Michigan</td>
<td>111.7</td>
<td>105.4</td>
</tr>
<tr>
<td>Missouri</td>
<td>53.5</td>
<td>64.9</td>
</tr>
<tr>
<td>Ohio</td>
<td>209.9</td>
<td>132.3</td>
</tr>
</tbody>
</table>

**Table 3.1:** The mean number of exhibition swine reported per county fair for Illinois, Indiana, Iowa, Michigan, Missouri and Ohio in 2013
Table 3.2: Descriptive statistics for continuous variables among survey participants.

Descriptive statistics are displayed for continuous variables collect from survey participants with exhibition swine during 2014. *This was collected from only a portion of the premises that reported having isolation as part of their farm management.
Table 3.3: Binary variables collected among survey participants. Displayed are the responses for survey participants concerning the on-farm management practices that they conducted with their 2014 exhibition swine. ¹For the farms that did not directly mix new swine into existing swine population, swine were placed in a separate pen that was not cleaned or disinfected (9.03% (25/277)), a separate cleaned pen (24.91% (69/277)) or a separate pen that was cleaned and disinfected (66.06% (183/277)). ²For the farms that obtained swine from an off farm source, the majority of swine were purchased between March and April (84.33% (296/351)). ³For the farms that raised other livestock at same location as exhibition swine the species reported were cattle (53.54%), goats (32.39%), poultry (32.62%), horses (27.30%), sheep (24.78%) and other, such as llamas, rabbits, etc., (11.70%).
<table>
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<tr>
<th>Activity</th>
<th>Farms responses</th>
</tr>
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<tbody>
<tr>
<td>Commercial swine production at same location as exhibition swine</td>
<td>Yes: 13.27% (56/422) No: 86.73% (366/422)</td>
</tr>
<tr>
<td>Hosted an open house/sale where attending people came into contact with</td>
<td>Yes: 14.59% (62/425) No: 85.41% (363/425)</td>
</tr>
<tr>
<td>swine during 2014</td>
<td></td>
</tr>
<tr>
<td>New swine were mixed directly into existing swine population on farm¹</td>
<td>Yes: 22.68% (93/410) No: 77.32% (317/410)</td>
</tr>
<tr>
<td>Exhibition swine were obtained from an off farm source²</td>
<td>Yes: 75.36% (315/418) No: 24.64% (103/418)</td>
</tr>
<tr>
<td>Other livestock raised at same location as exhibition swines³</td>
<td>Yes: 66.51% (282/424) No: 33.49% (142/424)</td>
</tr>
<tr>
<td>Swine from multiple farms were hauled to the fair together</td>
<td>Yes: 18.05% (76/421) No: 81.95% (345/421)</td>
</tr>
<tr>
<td>Other species were hauled to the fair with swine⁴</td>
<td>Yes: 7.35% (31/422) No: 92.65% (391/422)</td>
</tr>
<tr>
<td>Household members and/or participants have contact with swine other</td>
<td>Yes: 45.15% (191/423) No: 54.84% (232/423)</td>
</tr>
</tbody>
</table>
Table 3.4: Swine vaccination status for type A influenza and correlating results.

The vaccination status for incoming swine at nine agricultural fairs in Ohio and Indiana during 2014, and the test results for influenza A virus.
CHAPTER 4: Conclusions

This thesis began exploring IAV and the range of hosts that this diverse virus effects. The major human pandemics were reviewed and the role of novel IAV genes from spill-over into the human population from other species was studied. Swine in particular have been notated as a unique host that can allow for novel IAV entry into the human population. This zoonotic movement of IAV between swine and humans is most often found at locations with large human-swine interfaces, such as agricultural fairs. Exhibition swine and commercial swine have been previously identified as two separate swine populations. Little was known about the interaction between these two populations, though viral movement of IAV has been documented to take place between the two populations. There previously were few attempts to characterize the typical exhibition swine management practices and define where this swine population is geographically located in the United States. Thus two objectives were set out to be addressed in this thesis: 1. To determine the incoming prevalence of IAV among exhibition swine at agricultural fairs. 2. To characterize the on-farm management practices used for exhibition swine and determine if any management practices were associated with bringing IAV to agricultural fairs.

Chapter 2 of this thesis addresses the first objective though sampling individual swine as they arrived at agricultural fairs in Ohio and Indiana. Sampling revealed that the
prevalence of IAV was low at 1.5% among incoming exhibition swine, showing that IAV isolates can only be recovered from a few pigs entering into exhibitions. Furthermore, the corraling of swine through a chute during the entrance process at the fair was identified as a potential point for swine-swine transmission of IAV. Recognizing the low incoming prevalence of IAV at these exhibitions and the challenge subclinical infections pose to detection of this disease, it is proposed that mitigation strategies should be focused on limiting the spread of IAV during the fair.

The exhibition swine populations of this study were characterized in Chapter 3. The density maps of the estimated commercial and exhibition swine populations in our six participating states reflected that these two populations differ in geographical location. Participants reported showing their swine on average at 3.4 exhibitions, with one participant exhibiting at 50 exhibitions during 2014. This large movement between multiple swine populations differs from the typically limited movement observed in the commercial swine herds. Other on-farm management practices for exhibition swine, such as housing and biosecurity, were also different relative to the commercial swine industry. Interestingly, an interaction between the two swine populations was found with 45.4% exhibitors and/or household members having frequent contact other swine or the environment of swine. Additionally, both commercial and exhibition swine production was reported for 13.3% of the premises. Vaccination, while not always effective, was reported for 62.7% of the swine entering the exhibitions in this study. The only management practice significantly associated with the prevalence of IAV in pigs at arrival to fair was hosting an open house/sale on-farm (OR=3.93; CI: 1.10-13.06). It is
believed that the event of an open house/sale is does not directly affect the IAV status of the swine, but individuals that host open house/sales tend to be professional showmen and thus their swine would attend more exhibitions year round, allowing for more chances for IAV exposure.

This study has further demonstrated that exhibition swine are a unique swine population in the United States. They serve as an important host in the evolution and ecology of IAV for both the swine population as a whole and the human population. Limiting the spread of IAV in the exhibition swine host will improve both animal and human health.
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INFORMED CONSENT FOR PARTICIPATION IN RESEARCH ACTIVITIES
Swine Background Survey

I. INTRODUCTION/PURPOSE:
You are being asked to participate in a research study. The purpose of this study is to assess how exhibition swine are raised on farm prior to attending exhibition fairs. You are being asked to volunteer because you have taken part in the management of exhibition swine this show year of 2014. Your involvement in this study will begin when you agree to participate and will continue until you have completed the swine background survey.

II. PROCEDURES:
As a participant in this study, you will be asked to answer questions regarding the management of exhibition swine during this year, 2014. Your participation in this study will last for the time required to complete the swine background survey, estimated 5-8 minutes. Any information collected from this study will be collected anonymously. No personal identifying information will be written with responses to the questions asked.

III. RISKS AND BENEFITS:
Your participation in this study does not involve any significant risks and you have been informed that your participation in this research will not benefit you personally. There is no way for researchers to find out who you are, and your data will not be shared with any other parties under any circumstance.

IV. CONTACTS AND QUESTIONS:
The Ohio State University personnel have offered to and have answered any and all questions regarding your participation in this research study. If you have any further questions, you can contact Andrew Bowman at (614) 292-6923 or bowman.214@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

V. VOLUNTARY PARTICIPATION
You have been informed that your participation in this research study is voluntary. You may skip any questions that you do not want to answer. If you decide to take part, you are free to withdraw at any time. If you withdraw no more information will be collected from me. The above-named investigator has answered your questions and you agree to be a research participant in this study.

You will be given a copy of this consent form to keep.
Swine Background Survey 2014
The Ohio State University

1. Is there any commercial swine production located at the same farm as these show pigs? If yes what type of production?
   - Yes, nursery
   - Yes, breeding he- sows and piglets
   - Yes, breeding boars
   - Yes, grower-finisher
   - No

2. How many swine were on farm January 1st 2014? Number of Swine: ____________

3. How many swine were on farm yesterday? Number of Swine: ____________

4. What was the maximum number of swine on farm between January 1st and now? Number of Swine: ____________

5. Do household members and/or exhibitors have contact with swine or the environment of swine, other than their own at least once a week?
   - Yes: household members
   - Yes: participants
   - Yes: both
   - No

6. How many exhibitions have swine from this farm attended during 2014? Number of Exhibitions: ____________

7. How many swine have returned to this farm from exhibitions? Number of Exhibitions: ____________

8. As of January 1th 2014 has this farm host an open house/sale where attending people would come into contact with the swine?
   - Yes
   - No

9. How were these show pigs housed on farm?
   - Total confinement with mechanical ventilation
   - Open building with natural ventilation and no outside access for swine
   - Open building with outside access for swine
   - Pasture with hut or no building
   - Isolation with prior mixing

10. When new swine are brought on farm are they mixed directly with existing swine population? If yes move to question 12
    - Yes
    - No

11. If new swine are not directly mixed with existing swine what steps are taken to prepare the area/pen for the new swine?
    - Swine pens or areas not cleansed and disinfected
    - Swine pens and areas not cleaned and disinfected

12. In which county were the show pigs for this exhibition raised? County: ____________ State: ____________

13. Were the show pigs for this exhibition obtained from an off farm source?
    - Yes
    - No

14. What month were show pigs for this exhibition purchased, if obtained from an off farm source? Month: ____________

15. To the nearest mile, how far is it to the nearest commercial swine farm from your show pig facility?
    - Commercial swine on farm
    - <1 mile
    - 1-2.9 miles
    - 2-3.9 miles
    - 4-5.9 miles
    - 6-7.9 miles
    - 8-10 miles
    - >10 miles

16. In regards to prior question, Do you know what stage swine is raised there?
    - Not known
    - Sow breeder
    - Boar breeder
    - Nursery
    - Grower-finisher.
17. Is there isolation for swine returning to farm from exhibition?
   □ No
   □ Yes, in separate pen
   □ Yes, in separate barn
   □ Yes, at separate location
   □ Isolation time (Day:Hours): __________

18. Are livestock other than swine kept at the same farm/ location as these show pigs?
   □ Cattle
   □ Poultry
   □ Sheep
   □ Goats
   □ Horses
   □ Other: __________

19. Before loading pigs, what preparation was done to the trailer/truck used to haul these show pigs to this exhibition? Check all that apply
   □ Organic material removed
   □ Rinsed with water
   □ Washed with detergent
   □ Disinfected
   □ ≥2 hours of drying time
   □ Supplementary heat application
   □ Clean bedding applied

20. Were swine from different farms hauled to this exhibition together? If so how many farms were hauled together?
   □ Yes, number of farms: __________
   □ No

21. What was the total time between loading on farm and unloading at this exhibition?
   Total Time (Hours: Mins): __________

22. Were other animals transported with these show pigs?
   □ Yes, please list other species: __________
   □ No

23. May we contact your veterinarian regarding recommended vaccinations pig vaccinations? If yes who is your veterinarian? Name: __________

24. Information on individual pig(s)

<table>
<thead>
<tr>
<th>Pig ID#</th>
<th>Vial #</th>
<th>Number of exhibitions attended</th>
<th>Number of exhibitions that will be attended</th>
<th>Date of last exhibition attended by this pig</th>
<th>Vaccinations received</th>
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