REVIEW

The Role of Swine in the Generation of Novel Influenza Viruses

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Impacts

- Swine can be a mixing vessel for human, avian and swine influenza A viruses.
- This mixing can lead to unique reassortant influenza A viruses being generated in swine.
- The unique viruses may infect humans.

Keywords:
Swine; mixing vessel; influenza A virus

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Summary

The ecology of influenza A viruses is very complicated involving multiple host species and viral genes. Avian species have variable susceptibility to influenza A viruses with wild aquatic birds being the reservoir for this group of pathogens. Occasionally, influenza A viruses are transmitted to mammals from avian species, which can lead to the development of human pandemic strains by direct or indirect transmission to man. Because swine are also susceptible to infection with avian and human influenza viruses, genetic reassortment between these viruses and/or swine influenza viruses can occur. The potential to generate novel influenza viruses has resulted in swine being labelled 'mixing vessels'. The mixing vessel theory is one mechanism by which unique viruses can be transmitted from an avian reservoir to man. Although swine can generate novel influenza viruses capable of infecting man, at present, it is difficult to predict which viruses, if any, will cause a human pandemic. Clearly, the ecology of influenza A viruses is dynamic and can impact human health, companion animals, as well as the health of livestock and poultry for production of valuable protein commodities. For these reasons, influenza is, and will continue to be, a serious threat to the wellbeing of mankind.

Introduction

The ecology of influenza A viruses is very complicated involving multiple host species and viral genes. Traditionally, the taxonomy of this group of viruses has been based on the antigenic properties of 2 surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). There are 16 HA and 9 NA subtypes that have been found naturally paired in many combinations: H1N1, H3N2, H5N1, H7N7, etc. Avian species have variable susceptibility to influenza A viruses and wild aquatic birds are the reservoir for this group of viruses. When coupled with HA-NA gene permutations, there is an extraordinary opportunity for these viruses to change as they spread among birds. Occasionally, influenza A virus is transmitted from birds to mammals, which can lead to the development of human pandemic strains by direct or indirect transmission to man. The focus of this review will be the role of swine in the ecology of influenza A viruses, but it will not cover in depth their biology, nor the fascinating interplay of these viruses among different avian species. A list of recent reviews discussing these subjects is included to provide the reader with further supportive information.

Virology

Genomic organization of influenza A virus

Influenza A viruses are enveloped, single stranded RNA viruses belonging to the family *Orthomyxoviridae* (Lamb and Krug, 2007), which contains 4 other members: influenza B virus, influenza C virus, thogotovirus and isavirus. The genome of influenza A virus is about 13.6 kb and contains 8 negative sense segments, PB1, PB2, PA, HA, NP, NA, M and NS that encode 10 or 11 proteins. A ribonucleoprotein (RNP) complex that participates in RNA replication and transcription is formed between viral RNA segments and 4 proteins; the nucleoprotein (NP) and the three viral polymerase subunits (PB1, PB2 and PA) (Lamb and Krug, 2007). The viral glycoproteins HA and NA are highly variable, located on the virion surface and are the main targets of the host humoral immune response. The HA protein enables the virion to attach to sialic acid (SA)-containing molecules on the host cell surface and fuses to the host cell membrane to release the viral RNP complexes (Skehel and Wiley, 2000). Conversely, the NA protein removes SA to liberate newly synthesized viruses from infected cells (Gottschalk, 1957; Palese et al., 1974). Thus, efficient virus replication requires the balance of HA receptor-binding specificity and NA sialidase activity (Baum and Paulson, 1991; Kaverin et al., 2000; Wagner et al., 2000). Segments 7 and 8 each encode two proteins through differentially spliced transcripts, the matrix (M) and non-structural (NS) proteins, respectively. The M2 proteins are integrated into the viral envelope and serve as an ion channel (Wang et al., 1993). The M1 protein lies beneath the envelope and is thought to function in assembly and budding. NS1, a non-structural protein, is expressed only in infected cells during viral replication and has multiple functions (Krug et al., 2003). One such function is to counteract the interferon response of the host (Fernandez-Sesma, 2007). NS2 protein, originally designated as a non-structural protein, has been demonstrated to be a part of the influenza virion (Richardson and Akkina, 1991) and acts as a nuclear transport factor (O'Neill et al., 1998). PB1-F2, a protein encoded by an alternative ORF of the PB1 segment (Zell et al., 2007), is a pro-apoptotic polypeptide (Chanturiya et al., 2004) found in many influenza A viruses which can contribute to viral pathogenesis (Zamarin et al., 2006).

Depending on the virus and the species, the clinical outcome of influenza A infection in avian species is highly variable ranging from subclinical intestinal infection to systemic disease resulting in death. In land and aquatic mammals, influenza A viruses cause respiratory disease that can be fatal (Lamb and Krug, 2007). Although influenza B and C viruses are primarily human pathogens, influenza C can occasionally infect pigs and dogs (Ohwada et al., 1987). From a clinical perspective, the most significant characteristic of influenza A virus is its enormous variability. Two major mechanisms contribute to this: antigenic drift and antigenic shift. Antigenic drift is the accumulation of random mutations in viral genes due to low fidelity of the viral RNA polymerase. Recent research suggests that antigenic drift may have been responsible for the multiple waves of influenza disease and death during the infamous Spanish Flu pandemic of 1918–19 killing approximately 40∼100 million people worldwide (Taubenberger, 2006). Additionally, the segmented nature of the genome contributes to antigenic shift or reassortment. Reassortment occurs when different influenza viruses infect the same individual cell and exchange viral RNA segment(s), resulting in the generation of new viruses with a novel combination of genes. The Asian Flu pandemic of 1957 and the Hong Kong Flu pandemic of 1968 were a result of antigenic shift (Kawaoka et al., 1989).

Interaction between HA and sialic acid receptors

Attachment of influenza A virus to a host cell is mediated through a binding pocket on the HA and specific molecular species of SA linked to galactose on the host cell (Suzuki, 2005). The amino acid residues that form the binding pocket determine receptor specificity for H1, H2 and H3 viruses (Reviewed by Neumann and Kawaoka, 2006) and interact with SA that are normally classified into 2 major species [*N*-acetylneuraminic acid (NeuAc) or *N*-glycolylneuraminic acid (NeuGc)] according to the modification of the c-5 amino group (Suzuki et al., 1997). The SAs are linked to galactose by α2,3 linkage (α2,3 Gal) or an α2,6 linkage (α2,6 Gal) and the distribution of specific SAs expressed on cell surfaces varies among animal species. For example, bovine, equine and swine tissues possess both NeuAc and NeuGc, whereas human tissues possess only slight concentrations of NeuGc (less than 0.1% of total SA) (Suzuki et al., 2000). Influenza A viruses also differ in their recognition of NeuAc, NeuGc and 9-*O*-Ac-NeuAc (Higa et al., 1985). Most influenza A viruses isolated from humans preferentially recognize NeuAcα2,6Gal, whereas most avian isolates preferentially recognize NeuAcα2,3Gal (Rogers and Paulson, 1983). This apparent host preference has been mapped to specific amino acid positions. For H1 viruses, aspartate at position 190 (Asp-190) in the HA was found in human virus isolates, whereas glutamate at position 190 (Glu-190) was found in avian virus isolates. Asp or Glu at position 190 in the viral HA determines preferential binding to α2,6 or α2,3 linkages, respectively (Matrosovich et al., 2000; Gamblin et al., 2004; Kobasa et al., 2004; Stevens et al., 2004). Gln-226 found in avian viruses

determines specificity for SAα2,3Gal, whereas Leu-226 in human H2 and H3 viruses correlates with preferential binding to SAα2,6Gal (Rogers et al., 1983a; Matrosovich et al., 2000). In all human viruses except for the few early isolates from the 1957 Asian influenza outbreak (Matrosovich et al., 2000), Leu-226 is associated with Ser-228, whereas Gln-226 is associated with Gly-228 in avian viruses. NeuGcα2,3Gal recognition is essential for viral replication in ducks (Ito et al., 2000) and in horses (Suzuki et al., 2000). Swine viruses bind equally to both NeuAcα2,6Gal and NeuAcα2,3Gal or with a slight predominance towards NeuAcα2,6Gal (Ito et al., 1997; Suzuki et al., 1997). The finding that the predominant receptor on epithelial cells in the human respiratory tract was SAα2,6Gal-terminated glycoconjugates (Baum and Paulson, 1990) and SAα2,3Gal-terminated glycoconjugates on intestinal epithelial cells of ducks (Ito et al., 1998; Suzuki, 2005) combined with the above described virus binding preferences led to the hypothesis that the type of linkage of SA molecules to galactose contributes to the host range restrictions of influenza viruses. However, both 'human' (α2,6) and 'avian' (α2,3) receptors have been detected in the pig trachea (Ito et al., 1998; Suzuki et al., 2000), respiratory tract of humans (Shinya et al., 2006) and in the trachea and intestine of quail (Wan and Perez, 2006). These recent findings suggest the host restriction of some influenza A viruses is not as straight forward as once thought.

Epidemiology

Historical perspective on swine influenza in North America

All 16 HA and 9 NA subtypes of Influenza A viruses have been isolated from wild waterfowl and seabirds (Webster et al., 1992; Fouchier et al., 2005). Therefore, birds have been considered the reservoir for influenza A viruses from which novel viruses can emerge to infect mammalian species (Webby and Webster, 2001). Influenza A viruses infecting domestic poultry can be divided into two distinct groups based on their clinical manifestations in chickens: highly pathogenic avian influenza virus (HPAIV) and low pathogenic avian influenza virus (LPAIV). To date, all HPAIV isolates have been H5 or H7 subtypes and they can cause up to 100% mortality in chickens (Alexander and Brown, 2000). Only a limited number of subtypes of influenza A virus have been established in mammals. For example, only viruses of H1, H2, H3, N1 and N2 subtypes have circulated widely in the human population (Webster et al., 1992; Alexander and Brown, 2000), only H7N7 and H3N8 subtypes are found in horses (Webster, 1993; Wilson, 1993; Alexander and Brown, 2000) and only H1, H3, N1, and N2 subtypes have been consistently isolated from pigs (Webster et al., 1992; Olsen, 2002; Landolt and Olsen, 2007).

Swine influenza was first recognized clinically in pigs in the Midwestern US in 1918 (Koen, 1919) coinciding with the human influenza pandemic known as the Spanish flu (Webster, 2002). Since then, swine influenza has become important to the swine industry throughout the world (Olsen, 2002). The first swine influenza virus isolated from pigs in 1930 belonged to the H1N1 lineage of swine influenza viruses (Shope, 1931). Swine influenza virus induces clinical disease in swine similar to flu in humans, making it an important model to study influenza pathogenesis in a natural host. Specifically, swine influenza virus infections are manifested as acute respiratory disease characterized by fever, inactivity, decreased food intake, respiratory distress, coughing, sneezing, conjunctivitis and nasal discharge (McQueen et al., 1968; Alexander and Brown, 2000; Richt et al., 2003). The disease incubation period is between 1 and 3 days with rapid recovery beginning 4–7 days after onset. In a herd, swine influenza is characterized by high morbidity (approaching 100%) and generally low mortality (<1%) rates. Macroscopically, swine influenza virus-infected lungs display a purple-red, multifocal to coalescing consolidation of predominantly the cranioventral portions of the lung. Microscopic changes in the lung consist of necrosis of bronchiolar epithelial cells and sloughing of these cells into the airway lumen, which often contains cellular debris, proteinaceous fluid and a few leucocytes. This necrosis is accompanied by peribronchiolar lymphocytic infiltration and interstitial pneumonia of variable severity. During recovery, the bronchiolar epithelium becomes proliferative and lymphocytic cuffs become more prominent. Influenza viruses are part of the porcine respiratory disease complex (PRDC), acting in concert with other pathogens such as *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumonia*, *Pasteurella multocida,* porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2) (Thacker et al., 2001; Ellis et al., 2004).

Current perspective on swine influenza in North America

Before 1998, only classical-swine H1N1 viruses (cH1N1) were isolated from the US pig population (Easterday and Reeth, 1999). For nearly 70 years, swine influenza virus in North America was relatively stable with the cH1N1 as the only predominant subtype. In 1998, a severe influenza-like illness was observed in pigs on a farm in North Carolina with additional outbreaks in swine herds in Minnesota, Iowa and Texas. The causative agents for these outbreaks were identified as influenza A viruses of the H3N2 subtype. Genetic analysis of these H3N2 viruses

showed that at least two different genotypes were present. The initial North Carolina isolate was a double reassortant and contained gene segments similar to those of the classical-swine lineage (NS, NP, M, PB2, PA) combined with a recent human virus (HA, NA, PB1). The isolates from Minnesota, Iowa and Texas were triple reassortants containing gene segments from the classical swine virus (NS, NP, M) and the same human virus (HA, NA, PB1) in combination with an avian virus (PB2, PA) (Zhou et al., 1999). By the end of 1999, viruses antigenically and genetically related to the triple reassortant lineage were widespread in the US swine population (Webby et al., 2000), whereas the double reassortant virus did not spread efficiently among swine. The double and triple reassortant viruses contained similar HA genes with identical residues in critical receptor binding regions, suggesting that the success of the triple reassortant could not be explained just by variations within the HA and receptor binding. The major difference between the double and triple reassortant viruses was the acquisition of two avian polymerase genes (PA, PB2) in the triple reassortant H3N2.

Once established in the swine population, the H3N2 viruses evolved through genetic drift and reassortment with cH1N1 swine viruses. Currently, there are a number of reassortant viruses that have been identified including further H3N2 genotypes (Webby et al., 2000, 2004; Richt et al., 2003), H1N2 (Choi et al., 2002; Karasin et al., 2002), reassortant H1N1 (rH1N1) (Webby et al., 2004) and H3N1 viruses (Lekcharoensuk et al., 2006; Ma et al., 2006). The H3N2, rH1N1 and H1N2 viruses have become endemic and co-circulate in most major swine producing regions of the US and Canada. More recently, introductions of human-like H1 viruses that are genetically and antigenically distinct from the classical swine H1 lineage were identified in pigs in Canada (Karasin et al., 2006) and have spread across the US in swine herds (Gramer, 2007). One commonality among all of these reassortants is the maintenance of the internal genes (PB1 PB2, PA, NP M, NS) from the original triple reassortant virus. We refer to this constellation of genes as the triple reassortant internal gene (TRIG) cassette.

Molecular mechanism of host range restriction

In 1997, the emergence of a HPAIV subtype H5N1 (Shortridge et al., 1998) sparked a dramatic world-wide interest in influenza mainly because this virus is capable of infecting and killing humans. As part of the response to the H5N1, there has been a significant increase in resources available to study all aspects of influenza viruses. With the growing H5N1 database, it has become apparent that wild-bird populations are teeming in influenza A viruses. Accordingly, the transmission from wild birds to domesticated birds housed outside is a frequent event. What has been striking over the last 10 years is the relative ease with which the highly pathogenic H5N1 virus has affected wild- and domestic-bird populations throughout the Eastern Hemisphere along with many mammalian species including man (Webster et al., 2006; WHO, 2007b). Case reports have documented frequent direct transmission of the H5N1 virus from infected poultry to man (WHO, 2007a,b), a phenomenon once thought to be extremely rare. Prior to 1996, there were only 3 known cases of direct transmission of influenza A viruses to humans from birds (Campbell et al., 1970; Taylor and Turner, 1977; Webster et al., 1981).

Several viral proteins of influenza A viruses are known to be responsible for the host range restriction or species transmission. HA plays a key role in the restriction of interspecies transmission because HA binding to host cell receptors is a prerequisite for viral replication and transmission. The specificity of receptor molecules dominates viral entry into cells. HAs of AIVs preferentially bind to SAα2,3Gal-terminated saccharides on intestinal epithelial cells, whereas the HAs of human influenza viruses prefer SAα2,6Gal-terminated saccharides on tracheal epithelium (Rogers et al., 1983a,b). This difference may explain why AIV normally cannot replicate efficiently in man and conversely, why avian species are less susceptible to human influenza viruses. As the balance between HA receptor-binding affinity and NA receptor-destroying activity is critical for the efficient growth of influenza A viruses, NA also contributes to influenza viruses species specificity. Changes in HA receptor binding preference often result in alterations in the NA's SA substrate specificity (Landolt and Olsen, 2007). For example, after introduction of an AIV with an N2 NA into the human population, the SAα2,6Gal cleavage activity of the avian NA increased (Baum and Paulson, 1991; Kobasa et al., 1999), suggesting that the NA had adapted to the SAα2,6Gal receptor specificity of the HA (Neumann and Kawaoka, 2006). PB2 of the viral polymerase has been suggested to be critical for influenza virus replication and spread in mouse tissues (Subbarao et al., 1993; Hatta et al., 2001; Salomon et al., 2006). Normally, AIVs can infect mouse cells, but in contrast to mammalian viruses, do not replicate efficiently. This difference has been linked to the amino acid residue 627 of the PB2 gene where most avian viruses contain a glutamic acid in contrast to a lysine K residue found in mammalian viruses (Subbarao et al., 1993). The presence of 627K in the H5N1 virus isolates from humans in Asia (Hatta et al., 2001) and the H7N7 virus isolated from a fatal human case in the Netherlands (Fouchier et al., 2004) supports the assumption that this residue

may be a key host-range determinant. However, many of the Qinghai Lake avian H5N1 viruses contain 627K, indicating that this mutation is not restricted to mammals (Chen et al., 2006). Other genes, including PB1, NP, PA, M and NS1 are also reported to be involved in host range restriction of influenza viruses (Scholtissek et al., 1985, 2002; Tian et al., 1985; Snyder et al., 1987; Brown, 2000a), but their contributions seem to depend on a particular gene constellation of the influenza virus genome and interactions with cytoplasmic and nuclear host cell components.

Taking into account the human H5N1 cases, the recent genetic analysis of the reconstructed 1918 Spanish flu virus (these studies suggest that this virus may have been directly transmitted to man from an avian reservoir) (Taubenberger, 2006) and the 2003 H7N7 outbreak in the Netherlands that resulted in human infections (Fouchier et al., 2004), the direct transmission of avian influenza from avian species to man may be more common than once thought. Moreover, the direct transmission of influenza virus from bird to man is challenging the dogma that swine act as the critical link in the generation of pandemic influenza viruses affecting man.

Mixing vessel hypothesis

The 'mixing vessel' hypothesis was deduced by Scholtissek et al., (Scholtissek et al., 1985) who reasoned that swine infected with swine influenza virus could be dually infected with avian or human influenza A viruses. This dual infection could produce reassortants between swine and avian/human viruses. These reassortant viruses could then be transmitted to man resulting in the introduction of unique viruses into the human population. Occasionally, the combination of avian, swine and human genes could produce a human pandemic virus. This hypothesis was based on antigenic and genetic similarities between certain subtypes of avian, swine and human influenza viruses and the proven susceptibility of swine to infection with avian and human viruses. Support for this hypothesis was found at the molecular level with the discovery that avian and human influenza viruses have preferential binding to specific receptor types (avian viruses bind SAα2,3Gal-terminated saccharides and human viruses bind SAα2,6Gal-terminated saccharides) and these receptor types are differentially expressed between bird and human epithelia. In contrast, both receptor types can be found in the respiratory tract of swine (Ito et al., 1998). There are three parts to the mixing vessel hypothesis as discussed below: 1) swine are susceptible to avian and human influenza A viruses; 2) reassortment of swine/avian/human viruses occurs in the pig and 3) pigs can transmit reassortant influenza viruses to people.

Swine are susceptible to avian and human influenza A viruses

The isolation of wholly avian or human influenza A viruses from swine has confirmed the first part of the mixing vessel equation, i.e. the opportunity for introduction of new avian influenza genes into swine flu viruses via cross-species transmission. An H1N1 AIV was first detected in European swine in 1979, became well-established and is still circulating at present (Pensaert et al., 1981; Scholtissek et al., 1983). A different avian H1N1 virus was also transmitted to pigs in China in 1996 (Guan et al., 1996) and avian H4N6, H1N1 and H3N3 viruses have been isolated from Canadian pigs (Karasin et al., 2000a, 2004); currently, these viruses do not appear to be circulating in swine. Serological investigations in Asia detected antibody in pigs for avian H4 and H9 viruses (Ninomiya et al., 2002) as well as H5 viruses (Ninomiya et al., 2002; Choi et al., 2005). Although avian H9N2 (Xu et al., 2004; Cong et al., 2008; Shi et al., 2008; Yu et al., 2008a) and HPAIV H5N1 (Zhu et al., 2008) influenza viruses have been isolated from swine in China, they have not become established in swine. In addition to the above descriptions of natural infections of pigs with AIV, swine have been experimentally infected with H1-H13 AIVs (Kida et al., 1994) and may be susceptible to the H14-H16 virus subtypes as well. These field and experimental observations support the fact that swine can serve as an intermediate host for avian viruses.

Infection of pigs with wholly human viruses has been documented as well (Hinshaw et al., 1978; Bean et al., 1992; Brown, 2000b; Karasin et al., 2000b; Yu et al., 2007). The first confirmed case was the detection of the Hong Kong H3N2 virus from pigs in Taiwan (Kundin, 1970). Over time, Hong Kong H3N2 viruses were regularly isolated from pigs and serological studies detected subclinical infections throughout the world (Brown, 2000b). Interestingly, recent human-like H3N2 viruses have been isolated from pigs in southern China (Yu et al., 2008b). Human H1N1 viruses have been isolated from pigs (Nerome et al., 1982; Katsuda et al., 1995; Yu et al., 2007) and pig-to-pig transmission of human H1N1 viruses has been demonstrated under experimental conditions (Kundin and Easterday, 1972). Serological surveillance studies worldwide suggest that the prevailing human H1N1 strains are readily transmitted to pigs (Brown, 2000b). However, there are only a few reports of the isolation of wholly human H1 viruses from swine, suggesting that most strains are not readily transmitted among pigs in the field (Hinshaw et al., 1978). Although human-lineage influenza viruses have been isolated from pigs, sustained circulation of human viruses in pig populations was uncommon before 2005 (Hinshaw et al., 1978;

Ito and Kawaoka, 2000). However, since 2005, reassortant swine influenza viruses containing human-origin H1 and H1N2 gene segments have become established in the US (Gramer, 2006).

As with the maintenance of avian viruses in pigs, efficient transmission of human viruses among pigs requires mutational adaptation to the new host (Brown, 2000b; Lipatov et al., 2004).

Reassortment of swine/avian/human viruses occurs in the pig

Recently, full genomic analysis has confirmed the second part of the equation: the reassortment of avian, human and/or swine viruses occurs within swine. Although there is no direct evidence that the reassortment events leading to the 1957 or 1968 pandemic viruses occurred in pigs, genetic reassortment between avian H1N1 and human H3N2 viruses have occurred in European pigs (Castrucci et al., 1993; Brown et al., 1998) and the novel reassortant viruses, which contained mammalian HA and NA surface glycoproteins and the avian internal genes, transmitted to children in The Netherlands (Claas et al., 1994). Notably, similar reassortant H3N2 viruses were subsequently isolated from humans in Hong Kong (Gregory et al., 2001). Additional evidence supporting the 'mixing vessel' theory comes from the double (human/swine) and triple (avian/human/swine) reassortant viruses, H3N2, H1N2, rH1N1 and H3N1 that have emerged in US pigs since 1998, as described above (Zhou et al., 1999; Karasin et al., 2000b, 2006; Webby et al., 2000, 2004; Choi et al., 2002; Lekcharoensuk et al., 2006; Ma et al., 2006; Olsen et al., 2006). These triple reassortant H3N2, H1N2 and H1N1 viruses have become the predominant viruses circulating in the US swine population.

In the early 21st century, avian H9N2 and contemporary human H3N2 influenza viruses were found co-circulating in pigs in Southeastern China (Peiris et al., 2001). Recently, double reassortant H3N2 viruses containing human genes (HA and NA) and avian genes (PB2, PB1, PA, NP, M and NS) and the triple reassortant H3N2 viruses carrying human genes (HA and NA), a swine gene (NP) and avian genes (PB2, PB1, PA, M and NS) have emerged in pigs in China (Yu et al., 2008b). This suggests that genetic reassortment in pigs provides an opportunity to generate novel viruses and also provides further evidence that pigs serve as intermediate hosts for human and avian influenza viruses.

Importantly, a unique H2N3 influenza virus was recently isolated from clinically-affected pigs from two farms in the central US (Ma et al., 2007). Sequencing demonstrated that they were H2N3 influenza A viruses with 99.3–99.9% homology between the isolates. The HA segment was similar to an AIV H2N3 isolated from North American mallards and the NA sequence was similar to an AIV H4N3 isolated from North American blue-winged teal. The PA segment had high homology to an AIV H6N5 isolated from North American mallards and the remaining genes were similar to the US swine influenza virus TRIG cassette. Additionally, the avian-like H2 HA has an amino acid sequence in the receptor binding area that suggests preferential binding to the mammalian receptor. This mutation is identical to that in the initial reassortant human influenza isolates found at the beginning of the 1957 H2N2 pandemic. *In vivo* studies in swine, ferrets and mice, surrogate models for human infection, were conducted with the swine H2N3 virus. Experimentally infected pigs developed lung lesions following challenge and contact control pigs became infected and seroconverted. Furthermore, virus was able to transmit to contact ferrets from primary infected ferrets and even induce mortality in young mice. The only recognized common thread between the two pig farms was geographical location and the use of pond water for drinking and cleaning. The ability of the H2N3 viruses with avian origin surface glycoproteins to infect and replicate in 3 mammalian hosts without serial passage for adaptation in each species suggests that this virus is already adapted to the mammalian host. Moreover, H2 influenza viruses have been absent from human circulation since 1968 (Krauss et al., 2004; Liu et al., 2004; Munster et al., 2007) and individuals born subsequent to 1968 have little pre-existing immunity to this subtype. Therefore, they pose a potential human pandemic risk.

Pigs transmit reassortant influenza viruses to people

The third part of the equation, transmission of swine viruses to people, has been well documented (Reeth, 2007). In a recent review by Myers et al. (Myers et al., 2007), the authors report 50 cases of zoonotic swine influenza virus infections: 37 civilian cases and 13 military cases. A majority belonged to the H1N1 subtype, while a few were of the H3N2 subtype. The case-fatality rate of all reported cases was 14% (7/50). Civilian cases were described in the US (19 cases), Czechoslovakia (6 cases), the Netherlands (4 cases), Russia and Switzerland (3 cases each), Canada and Hong Kong (1 case each). The median age of the patients was 24.5 years and a majority of the patients (61%) reported a recent exposure to pigs, indicating that not all cases have a known direct contact with swine. For example, a well publicized outbreak of swine H1N1 virus caused one soldier to die and additional 12 to be hospitalized with respiratory illness at Fort Dix, New Jersey, in early 1976 (Gaydos et al., 1977). No evidence of exposure to pigs was ever found in this case.

Although swine influenza virus infections in humans without direct swine contact are possible, as expected, persons who work with swine are found to have an increased risk of zoonotic influenza virus infection (Myers et al., 2006). In this report, farmers, meat processing workers and veterinarians were demonstrated to have significantly elevated serological titers against H1N1 and H1N2 swine influenza virus compared with control subjects. Thus, occupational exposure may play an important role in the mixing vessel hypothesis.

Recent case reports confirm transmission from swine to humans is not a rare event. In the first case, an H3N2 swine influenza virus containing the TRIG cassette was isolated from a 7-month-old boy in Canada and believed to have been transmitted from human to human (Robinson et al., 2007). In the US, H1N1 swine influenza viruses containing the TRIG cassette were isolated from an individual in 2005 that may have become infected while butchering a hog (Newman et al., 2008) and from a parent and child in 2007 who were exposed to infected pigs at a county fair in 2007 (Swenson et al., 2008). Healthy pigs and people in close contact with sick pigs became clinically affected with an acute influenza-like illness. Swine influenza virus was isolated from several pigs and at least two people (parent and child). The viruses isolated from the humans were 100% identical to the pig viruses indicating that the virus was shared between pigs and people at the fair. In a swine infection model, this Ohio H1N1 virus was pathogenic, was shed at high titres and caused severe clinical disease (A. Vincent, S. Swenson, K. Lager, P. Gauger, C. Loiacono, Y. Zang, unpublished data). Subsequent to this case, almost identical H1N1 swine influenza viruses have been isolated from swine in several states indicating that this virus has spread. Case reports indicate at least a 10% mortality rate in finishing pigs (S. Henry, personal communication); human illness in these additional swine cases is not known.

Conclusion

The realization that humans and other mammals can frequently be infected with H5N1 HPAIV following exposure to infected birds has complicated the role of pigs as the mixing vessel in the transmission of avian viruses to man and the epidemiology of influenza A viruses. The physiological reason as to why certain viruses can directly infect man and others require an intermediate host is not fully understood. Certainly, the challenge dose may be a critical factor. In the case of the Ohio H1N1 swine influenza virus found at the livestock show (described above), the sick humans (children and adults) had sustained close contact with acutely-affected swine. The virus recovered from the sick pigs was demonstrated to induce severe pneumonia and was shed longer than other swine influenza viruses tested in a young pig challenge model (A. Vincent, S. Swenson, K. Lager, P. Gauger, C. Loiacono, Y. Zang, unpublished data). As most human H5N1 cases involved exposure to infected birds, it may be that these birds were slaughtered during peak shedding times, thus improving the odds for transmission. Additional factors could be the genetics of the infected host, potential recipient and virus. The likelihood of indirect transmission of influenza A viruses from an avian reservoir to man may be dependent on the intermediate host. For example, the efficient movement of H1, H2 and H3 virus subtypes may involve swine, while other subtypes might involve a different intermediate. Although HPAIV H5N1 viruses have been transmitted directly to man, it may be that some species of carnivores could act as an intermediate host as they are quite susceptible to infection with this subtype (Keawcharoen et al., 2004; Kuiken et al., 2004, 2006; Roberton et al., 2006; Amonsin et al., 2007; Thiry et al., 2007). Similar to pigs, quail have been shown to possess both the NeuAcα2,3Gal and NeuAcα2,6Gal receptors, suggesting that this bird could be an efficient mixing vessel as well for one or more subtypes. All these factors will require further study to determine their roles in the epidemiology of influenza A viruses.

The control of influenza in humans or animals is dependent on the rate of antigenic drift and shift; if there is limited change in the circulating viruses, then it is relatively easy to produce efficacious inactivated virus vaccines. However, if the rate of drift and shift accelerates, then it is difficult for vaccine production to keep up with circulating viruses. The mechanism that drives the rate of drift and shift is not fully understood, but like influenza A epidemiology, it is probably related to genetics of the virus and host. In the case of swine, empirical and experimental evidence demonstrates swine can generate novel influenza A viruses that have the potential to infect humans and some avian species. At present, it is difficult to predict which virus, if any, might induce a human pandemic. History would suggest that the likelihood of such an event is low; however, it seems prudent to minimize the risk of transmission of swine viruses to people as well as minimize the risk of transmission of novel viruses to swine. Thus, control and prevention strategies for swine influenza are warranted, not only from the standpoint of reducing economic losses for pork producers, but for public health purposes as well.

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