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**Review**

## The emergence of novel swine influenza viruses in North America<sup>\*1</sup>

Christopher W. Olsen

Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53706, USA

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**Abstract**

Since 1997, novel viruses of three different subtypes and five different genotypes have emerged as agents of influenza among pigs in North America. The appearance of these viruses is remarkable because there were no substantial changes in the overall epidemiology of swine influenza in the United States and Canada for over 60 years prior to this time. Viruses of the classical H1N1 lineage were virtually the exclusive cause of swine influenza from the time of their initial isolation in 1930 through 1998. Antigenic drift variants of these H1N1 viruses were isolated in 1991–1998, but a much more dramatic antigenic shift occurred with the emergence of H3N2 viruses in 1997–1998. In particular, H3N2 viruses with genes derived from human, swine and avian viruses have become a major cause of swine influenza in North America. In addition, H1N2 viruses that resulted from reassortment between the triple reassortant H3N2 viruses and classical H1N1 swine viruses have been isolated subsequently from pigs in at least six states. Finally, avian H4N6 viruses crossed the species barrier to infect pigs in Canada in 1999. Fortunately, these H4N6 viruses have not been isolated beyond their initial farm of origin. If these viruses spread more widely, they will represent another antigenic shift for our swine population, and could pose a threat to the world's human population. Research on these novel viruses may offer important clues to the genetic basis for interspecies transmission of influenza viruses.

**Author Keywords:** Influenza virus; Pig; Genetic reassortment; Interspecies transmission; Antigenic drift; Antigenic shift; Host range

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## 1. Introduction

Influenza was first recognized clinically in pigs during the late summer and fall of 1918 in the midwestern United States (Koen and Easterday), coincident with the dramatic and devastating human influenza pandemic that killed 20–40 million people around the world (Murphy and Webster, 1996). The first swine influenza viruses were isolated from pigs in 1930 (Shope, 1931). These were the progenitors of what is now recognized as the 'classical' H1N1 lineage of swine influenza A viruses. Influenza A viruses of other subtypes have been isolated relatively commonly from pigs elsewhere in the world, including H3N2 viruses (Sugimura; Nakajima; Ottis; Yasuhara; Mancini; Castrucci and Campitelli) and H1N2 viruses (Nerome; Gourreau; Brown; Brown; Ouchi; Ito and VanReeth). In addition, avian H1N1 viruses have been isolated from pigs in Europe and Asia (Pensaert; Schultz; Brown; Brown and Guan). However, from 1930 through the mid-1990s, influenza in North American pigs was caused almost exclusively by infection with classical H1N1 swine viruses. Serosurvey studies conducted in the United States in 1976–1977 (Hinshaw et al., 1978), 1988–1989 (Chambers et al., 1991) and 1997–1998 (Olsen et al., 2000) revealed high rates of seropositivity among pigs to classical H1 swine influenza viruses (28–51%), but much lower seroprevalence rates against H3 viruses. In fact, in the 1976–1977 (Hinshaw et al., 1978) and 1988–1989 (Chambers et al., 1991) studies, only approximately 1% of pigs had antibodies against H3 viruses, and prior to 1997, only three H3 viruses had been isolated from pigs in North America (Hinshaw; Bikour and Bikour). However, a dramatic shift in the epidemiologic pattern of swine influenza began in 1997–1998. The 1997–1998 serosurvey (Olsen et al., 2000) detected an unexpected and substantial increase in H3 seropositivity (8%), and H3N2 viruses began to be isolated from pigs in both the US and Canada during this time (Karasin and Zhou). Subsequently, reassortment between H3N2 viruses and classical H1N1 swine viruses led to the appearance of second generation H1N2 reassortant viruses (Karasin and Karasin). In addition, avian H4N6 viruses of duck origin have been isolated from pigs in Canada (Karasin et al., 2000b). This review summarizes what is known about these novel viruses, as well as recently described antigenic drift variants of classical H1N1 swine influenza viruses (Dea; Olsen; Olsen and Rekić), and discusses the potential veterinary and human public health implications of these viruses.

## 2. Background

Influenza A viruses are enveloped, negative-sense RNA viruses that encode 10 major viral proteins on eight independent segments of RNA. The hemagglutinin (HA or H) is a large N-linked glycoprotein that projects as trimers from the viral envelope. The HA binds to sialic acid-containing receptors and mediates infection of host cells and cell-to-cell fusion following proteolytic cleavage of the HA into HA1 and HA2 segments (Lamb and Krug, 1996). The HA1 forms the large globular head of the protein and contains the receptor binding site, as well as the major antigenic sites to which neutralizing antibodies are directed. In particular, antigenic sites designated Ca, Cb, Sa and Sb have been defined on both H3 and H1 HA proteins (Lubeck; Wiley; Winter; Caton and Raymond).

Neuraminidase (NA or N) is the second envelope glycoprotein. The tetrameric NA protein catalyzes cleavage of sialic acid from adjacent sugar residues. The NA may function to prevent virion aggregation and to enhance the release of budded virus particles (Lamb and Krug, 1996), and recent evidence suggests that the relative strength of HA binding and NA enzymatic activity function in concert to optimize virus replication (Rudneva and Hughes). In addition, NA may be a factor in influenza virus-induced apoptosis (Schultz and Morris) and it may function as a virulence factor by binding and activating plasminogen, thereby enhancing HA cleavage (Goto and Kawaoka, 1998). However, the overall role of NA in the virus life cycle remains controversial (Liu et al., 1995).

There are 15 different subtypes of HA and 9 different subtypes of NA that can be differentiated both antigenically and genetically, and it is their designations that define a virus as 'H1N1,' 'H3N2' and so on (Lamb and R). Historically, only a limited number of subtypes of influenza virus have been routinely isolated from individual mammalian species, e.g. H1N1, H2N2 and H3N2 from people, H3N8 and H7N7 from horses and H1N1, H3N2 and H1N2 from pigs. In contrast, waterfowl can be infected subclinically in their intestinal tracts with influenza viruses of all 15 HA and 9 NA subtypes. As such, they provide a vast global reservoir of influenza viruses in nature ( Webster; Webster and Hinshaw).

In addition to the HA and NA, influenza virions contain two other major structural proteins. The nucleoprotein (NP) encapsidates the viral RNA, while the M1 matrix protein interacts with both NP and the cytoplasmic tails of the HA and NA proteins to provide a structural framework for the virions. The M and NP also are the type-specific proteins that differentiate influenza A, B and C viruses (Lamb and Krug, 1996). An additional structural protein, M2, is an ion channel that functions early in the infection cycle to facilitate virus uncoating, and may function later in virus assembly to prevent premature pH-induced conformational changes in the HA ( Holsinger et al., 1994). The remaining influenza virus proteins are the polymerase proteins (PB1, PB2 and PA) that mediate viral RNA synthesis and the non-structural proteins (NS1 and NS2) which, along with NP and M, regulate RNA transcription and replication, RNA splicing and nuclear transport of RNA ( Lamb and Krug, 1996).

Influenza virus infections in pigs present in two clinical forms. Most commonly, infections present as epizootics of respiratory disease characterized by fever and lethargy, coughing, dyspnea and sometimes nasal or ocular discharge (Easterday and Hinshaw, 1992). In this epizootic form, the onset of illness is abrupt, the pigs are sick for 3–7 days and the outbreak progresses through an entire facility within 2–3 weeks ( Easterday and Hinshaw, 1992). The pigs may also be off-feed for as long as 1–2 weeks, which makes swine influenza an economic hardship for farmers trying to get the pigs to market weight ( Janke, 1998). In addition to this epizootic form of disease, influenza virus infections also contribute to a more insidious condition known as the porcine respiratory disease complex, acting in concert with porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae* and bacterial agents of pneumonia (Halbur, 1996).

Influenza viruses undergo two major forms of evolution, antigenic drift and antigenic shift. Antigenic drift refers to the gradual accumulation of point mutations in viral proteins (principally the HA) in response to immune pressure in the population (Murphy and Webster, 1996). Antigenic drift is a well-described phenomenon among human influenza viruses and is the reason for yearly re-evaluation of the human influenza vaccines and periodic replacement of vaccine viruses with more contemporary strains. Historically, the rate of drift among swine influenza viruses was thought to be significantly slower than that among human viruses ( Raymond; Luoh and Murphy), but it is now clear that antigenic drift also occurs among swine viruses ( Dea; Olsen; Olsen and Rekik).

Antigenic shift is a more dramatic form of genetic and antigenic change in which viruses of a new subtype begin circulating within a given population. Antigenic shifts may occur because of interspecies transmission of viruses in toto or through genetic reassortment between a virus currently circulating in a population and one or more new viruses (Murphy and Webster, 1996). With eight independent segments of RNA, it is possible to obtain 256 different possible genotypes from two parental viruses, which makes reassortment a very powerful mechanism for generating genetic diversity. Both methods of antigenic shift have been documented among human influenza viruses. The introduction of H5N1 ( Claas; Subbarao and Mounts) and H9N2 ( Guo; Peiris and Lin) influenza viruses into the human population of southeast Asia in 1997 and 1999 were examples of in toto interspecies transmissions of avian viruses. Fortunately, these viruses did not spread efficiently from person-to-person, and thus a population-wide antigenic shift and influenza pandemic did not occur. In contrast, the human influenza pandemics of 1957 and 1968 ( Fig. 1) occurred following reassortment of contemporary human influenza viruses and avian influenza viruses (Webster et al., 1992). It is unlikely that these reassortment events occurred directly in either people or birds. Avian influenza viruses preferentially bind to cells using receptor molecules with an  $\alpha 2,3$  linkage between sialic acid and galactose (present in intestinal

epithelial cells in ducks, but largely lacking in the human respiratory tract), whereas human influenza viruses prefer receptors with a  $\alpha$ 2,6 sialic acid-galactose linkage (present in tracheal epithelial cells in humans, but lacking in the avian intestinal tract) (Rogers; Rogers; Rogers; Connor; Ito; Ito and Ito). As a consequence, human influenza viruses do not replicate efficiently in birds, and vice versa (Hinshaw; Beare; Webster; Murphy and Scholtissek). In contrast, pigs are uniquely susceptible to infection with human and avian influenza viruses because their tracheal epithelial cells contain both  $\alpha$ 2,3 and  $\alpha$ 2,6 receptors (Ito and Ito). As such, pigs have been hypothesized to be the "mixing vessel" hosts for human-avian virus reassortment (Scholtissek; Scholtissek and Webster). In support of this theory, human-avian virus reassortants have been isolated from commercially-raised pigs in Europe and, thereafter, from children in the Netherlands (Castrucci and Claas).

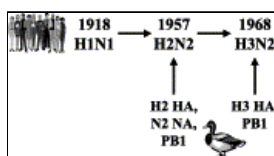


Fig. 1. Schematic representation of the genetic reassortment events that lead to the development of the 1957 and 1968 pandemic strains of human influenza A viruses. In each case, an avian influenza A virus provided new HA and PB1 (and in 1957, NA) genes during reassortment with the existing human influenza A viruses to produce antigenic shift variant viruses.

### 3. Variants of classical H1N1 swine influenza viruses

Research by Sheerar and Luoh and Noble et al. (1993) demonstrated that the classical H1N1 swine influenza viruses in the United States remained antigenically and genetically highly conserved from 1965 through the 1980s. In fact, the rate of drift in the HA1 segments for swine viruses was 0.4–0.48% amino acid changes/year (Luoh et al., 1992), compared with a rate of 0.8% for human H1N1 viruses (Raymond et al., 1983). More recently, however, a number of antigenic and genetic variants have been documented among H1 swine viruses. A virus isolated from a pig in Nebraska in 1992, A/Swine/Nebraska/1/92 (Sw/NB/92) (Olsen et al., 1993), failed to react in hemagglutination-inhibition (HI) assays with 2 of 4 monoclonal antibodies (Mabs) used to characterize classical H1 swine influenza viruses. This virus contained 18 amino acid mutations in the HA1 segment compared with A/Swine/Indiana/1726/88 (Sw/IN/88), a prototypical H1N1 swine influenza virus isolated just 3 years earlier (Luoh et al., 1992). These mutations included three changes in the Ca and Sb antigenic sites and a mutation adding a potential glycosylation site (Olsen et al., 1993). However, this virus is of particular interest because not only was it antigenically and genetically atypical, but affected pigs also displayed somewhat atypical clinical signs. The naturally infected pigs had persistent, high (up to 42 °C) fevers, but only mild evidence of respiratory disease and minimal coughing (Olsen et al., 1993). Similarly, a virus isolated in Quebec in 1991, A/Swine/QC/192/91 (Sw/QC/91) (Dea and Rekik), was also both antigenically and genetically divergent and induced atypical lung pathology. In contrast to the bronchopneumonia that typifies classical swine influenza, pigs infected naturally or experimentally with Sw/QC/91 developed a proliferative pneumonia with extensive macrophage invasion of the lung parenchyma, proliferation of type II pneumocytes and thickening of the alveolar septae (Dea et al., 1992). This virus contained 15 amino acid mutations in its HA1 segment compared with Sw/IN/88, including 2 changes in antigenic sites Ca and Sb, as well as a mutation eliminating a potential glycosylation site (Rekik et al., 1994). Interestingly however, Sw/NB and Sw/QC only shared four mutations in common.

Most recently, substantial antigenic drift has been documented among classical H1N1 influenza viruses isolated from slaughter pigs in the north-central United States between

September 1997 and August 1998 (Olsen et al., 2000). Twenty six viruses could be divided into seven antigenic groups based upon patterns of Mab reactivity in HI assays, and none of the viruses reacted similar to the Sw/IN/88 reference virus. A subset of these viruses (11 isolates) were also subjected to HA gene sequence analysis, which revealed 3–5% nucleotide and 2–5% amino acid divergence compared with Sw/IN/88. Outside of the United States, de Jong et al. (1999) have also demonstrated antigenic drift among H3N2 swine influenza viruses in The Netherlands.

What are the implications for antigenic drift among the classical H1N1 swine influenza viruses? The attributes of infection with Sw/NB/92 and Sw/QC/91 suggest that the clinical and pathologic descriptions of swine influenza may need to be expanded to include patterns associated with atypical viruses. In addition, one question should be whether antigenic drift has occurred to an extent that necessitates updating of available vaccines with contemporary strains? The answer at this time appears to be no, because although the 26 viruses isolated in 1997–1998 were antigenically divergent from each other and Sw/IN/88 when assessed by Mab analysis, they and Sw/IN/88 all reacted to equally high titers with serum from pigs immunized with a commercial H1N1 swine influenza vaccine (Olsen et al., 2000). It will, however, be very important to continue to monitor antigenic drift in the future and to continually reassess this vaccine issue. It would be ideal if a group of geographically diverse diagnostic laboratories throughout North America and elsewhere in the world would develop a standardized set of reagents and protocols for assessing antigenic drift in swine influenza viruses. These reference laboratories could then meet yearly or biennially to address antigenic drift and vaccine composition, as occurs for human influenza viruses.

#### 4. The emergence of H3N2 influenza viruses among pigs in North America

The role that pigs play as the mixing vessel hosts for genetic reassortment among human and avian influenza viruses and the development of new pandemic human influenza viruses is well recognized. In addition, it is clear that swine influenza viruses can be transmitted to people as zoonotic agents (Smith; Top; Hinshaw; Eason; Dasco; Patriarca; de; Rota; Wentworth; Wentworth; Kimura; Cooper and Olsen). However, the lesson learned over the past several years is that human influenza viruses can also been transmitted to pigs as reverse zoonotic infections, and that reassortment in pigs can then produce viruses of substantial clinical concern for the pigs themselves. Since 1997, three different genotypes of H3N2 viruses have been isolated from pigs in North America ( Fig. 2). In January 1997, a non-reassortant human H3N2 virus (Sw/ONT/97) was isolated from a 1-week-old piglet in Ontario that had died of pneumonia (Karasin et al., 2000c). In August 1998, a reassortant H3N2 virus containing HA, NA and PB1 genes of human influenza virus origin and M, NP, NS, PB2 and PA genes of classical swine influenza virus origin was isolated from a pig in North Carolina (Sw/NC/98). Disease in affected pigs was characterized by not only typical respiratory disease, but also high fevers, abortions and deaths among sows ( Zhou et al., 1999). Finally, triple reassortant viruses containing HA, NA and PB1 genes of human influenza virus origin, M, NP and NS genes of classical swine influenza virus origin and PA and PB2 genes of avian influenza virus origin also emerged in 1998 ( Zhou and Karasin). Serosurveillance among pigs in the north-central United States confirmed an increase in H3 virus infection among pigs during this same time period ( Olsen et al., 2000). Following the earliest documented appearance of the triple reassortant viruses among pigs in Nebraska in March 1998 ( Karasin et al., 2000c), published reports have confirmed isolation of these viruses from pigs in Minnesota, Iowa, Texas, Illinois, Wisconsin, Oklahoma, North Carolina and Colorado ( Karasin; Webby and Zhou). In addition, the USDA National Veterinary Services Laboratory has found that 27% of swine influenza viruses isolated between 1 October 2000 and 30 April 2001 were H3N2 subtype (S. Swenson, personal communication) and a more recent serosurvey (1998–1999) revealed a 20.5% H3 seroprevalence among pigs in 23 US states ( Webby et al., 2000).

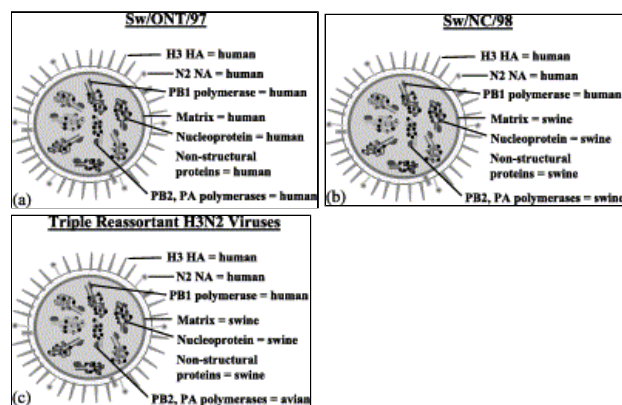


Fig. 2. Schematic representations of the genotypes of H3N2 influenza A viruses isolated from pigs in North America since 1997. The Sw/ONT/97 virus (Fig. 2a) was a wholly human influenza A virus that crossed the species barrier from a person to a pig without reassortment. In contrast, the Sw/NC/98 (Fig. 2b) was a reassortant between human and classical swine influenza A viruses, and the triple reassortant viruses (Fig. 2c) were reassortants between human, swine and avian influenza A viruses.

It is interesting to note that, while the triple reassortant viruses have spread widely through the swine population, to the author's knowledge, Sw/ONT/97- and Sw/NC/98-like viruses have not been isolated again since their initial appearances on their farms-of-origin. It is possible that this is simply due to differences in the biosecurity of the farms-of-origin of these viruses or differences in animal movement subsequent to the initial infections. However, it has also been postulated that genetic differences among these H3N2 viruses may impact their infectivity and replication efficiency in pigs (Zhou and Karasin). Host-range restriction among influenza viruses is considered to be a polygenic trait and evidence exists for contributions by multiple genes (Webster and Murphy). Nonetheless, the HA is thought to be a major host range factor since it is the receptor binding protein. The initially described triple reassortant H3N2 viruses contained at least 12 amino acid differences as compared with the HAs of the 1995-lineage human H3 viruses that they were most closely related to phylogenetically (Karasin and Zhou), including three amino acid residues within the receptor bind site and loss of a potential N-linked glycosylation site. In contrast, the double reassortant Sw/NC/98 and wholly human Sw/ONT/97 viruses lacked all but one to three of these differences, suggesting that these may represent swine adaptation mutations. However, sequence analysis of additional triple reassortant viruses described subsequently by Webby et al. (2000) has revealed that a subset of these viruses also lacked some of these HA differences (Olsen, unpublished results). Beyond the HA protein, the triple reassortant viruses also had 9–13 differences in NA compared with the phylogenetically closest human influenza viruses (Zhou et al., 1999), and the presence of avian polymerase genes or the overall constellation of human, swine and avian internal protein genes functioning together may enhance virus replication in pigs (Zhou et al., 1999).

There are several important considerations related to the emergence of the H3N2 viruses. An obvious implication is the need to include these viruses in routine swine vaccination regimes. An H3N2 swine influenza virus vaccine is now commercially-available and work is underway to develop bivalent vaccine products. However, an unexplained observation is that among pigs that are simultaneously vaccinated with monovalent H1N1 and H3N2 vaccines, some animals seroconvert poorly or not at all to the H1N1 virus (R. Fleck, personal communication; Scatozza et al., 1995). A second implication relates to the overall epidemiology of influenza among North American pigs. It remains to be determined whether both H3N2 and H1N1 viruses will continue to co-circulate, as is happening now, or whether one virus will eventually predominate. When the antigenic shifts from H1N1 to H2N2 and from H2N2 to H3N2 occurred among human influenza viruses in 1957 and 1968, the pre-existing viruses disappeared from the human population, whereas both H3N2 and H1N1 viruses have circulated since the re-introduction of H1N1 viruses in 1977 (Murphy and Webster, 1996). Finally, given the widespread co-circulation of H3N2 and H1N1 viruses

among pigs since 1998, it is not surprising that second generation reassortant viruses have already begun to emerge. These include the H1N2 viruses described below, as well as an H1N1 virus isolated from a man in Wisconsin in 1998 that proved to be a reassortant between one of the triple reassortant H3N2 swine viruses and a classical H1N1 swine virus (Cooper et al., 1999).

### 5. H1N2 influenza viruses in pigs in the United States

In November 1999, influenza-like respiratory illness, as well as abortions in sows, occurred among pigs on a farm in Indiana. An influenza virus isolated from lung tissue of a sow that died during the outbreak was shown by HI and neuraminidase-inhibition (NI) antigenic assays and genetic analyses to be an H1N2 subtype virus (Karasin et al., 2000a). Further sequencing and phylogenetic analyses revealed that this was a second generation reassortant virus with NA, PB1, M, NP, NS, PA and PB2 genes derived from a recent triple reassortant H3N2 virus and an H1 HA closely related to the HAs from classical H1N1 swine influenza viruses isolated in 1997–1998 (Fig. 3) (Karasin et al., 2000a). To the author's knowledge, this was the first H1N2 virus ever isolated from pigs in the United States. However, H1N2 viruses had been isolated previously from pigs in Japan in 1978–1980 and 1989–1992 (Nerome; Ouchi and Ito), in France in 1987–1988 (Gourreau et al., 1994), in the United Kingdom since 1994 (Brown and Brown) and in Belgium in 1999 (VanReeth et al., 2000). The H1N2 viruses in Japan and the United Kingdom caused widespread outbreaks of disease. Likewise, since the initial report of the H1N2 virus in Indiana, additional H1N2 viruses have been isolated from pigs elsewhere in Indiana, and in Minnesota, Ohio, Iowa, Illinois and North Carolina (Karasin et al., 2002). As such, it appears that H1N2 viruses are now circulating among pigs in the United States together with H1N1 and H3N2 viruses. Therefore, if the overall epidemiology of swine influenza is to be documented and understood, it is increasingly important that diagnostic laboratories characterize swine influenza viruses as to both HA and NA subtype. If viruses are only subtyped by their HA, it will not be possible to distinguish H1N1 from H1N2 viruses. In addition, beyond the appearance of H1N2 viruses, it is possible, if not likely, that H3N1 viruses will also emerge. While conventional HI and NI assays remain as viable methods to subtype influenza viruses, these techniques may soon be replaced by genetic methods. The author's laboratory now subtypes all viruses by HA and NA gene RT-PCR assays, and recent developments in multiplex RT-PCR and microarray technology (Zou and Li) herald likely approaches for the future.

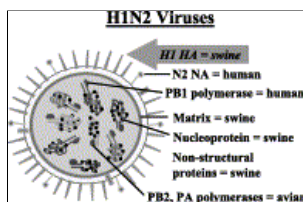


Fig. 3. Schematic representation of the genotype of the H1N2 influenza A viruses isolated from pigs in the United States since 1999. Genetic and phylogenetic analyses indicated that these viruses were derived by reassortment between a triple reassortant H3N2 virus and a classical H1 swine virus. The classical swine virus contributed an H1 HA RNA segment, whereas all of the remaining gene segments originated from the triple reassortant H3N2 virus.

### 6. Interspecies transmission of avian H4N6 influenza viruses to pigs in Canada

Since the respiratory tracts of pigs contain both  $\alpha 2,3$  and  $\alpha 2,6$  sialic acid receptors (Ito et al., 1998a), the species barrier to transmission of avian influenza viruses is relatively less stringent for pigs than for people. Kida et al. (1994) has demonstrated that pigs can be experimentally infected by a wide variety of subtypes of avian influenza viruses.

Nonetheless, relatively few examples of natural infection of pigs with wholly avian influenza viruses have been documented. The most widespread infections have occurred in Europe. H1N1 viruses of avian origin became a dominant cause of swine influenza in Europe following their introduction into the pig population in the late 1970s ( [Pensaert; Scholtissek; Webster; Brown and Brown](#)) and avian H1N1 viruses were also isolated from pigs in Asia in 1993 ( [Guan et al., 1996](#)). In North America, a serosurvey in the northern Midwest region of the United States in 1997–1998 detected seropositivity to an avian H1N1 virus (A/Duck/Alberta/35/76) in a small percentage of pigs tested ( [Olsen et al., 2000](#)), although avian H1N1 viruses were not isolated from pigs during that study.

The most recent example of infection of pigs with an avian influenza virus occurred on a large commercial swine farm in Ontario, Canada in October 1999. Genetic and antigenic analyses demonstrated that viruses isolated from pigs during a 3-week-long outbreak of respiratory disease were wholly avian H4N6 viruses ( [Karasin et al., 2000b](#)). The source of this virus was presumed to be ducks on an adjacent lake from which the farm drew water and on which waterfowl commonly congregated in preparation for southward migration each fall. Viruses with H4 and/or N6 surface glycoproteins are among the most common influenza viruses in the Canadian duck population ( [Sharp et al., 1993](#)), and because of the high level of virus shedding by ducks, influenza viruses have been previously isolated directly from unconcentrated lake water ( [Laver et al., 2000](#)).

Follow-up serologic screening has shown that this H4N6 virus spread to additional units of the original farm, suggesting that it has the ability to spread from pig-to-pig (Olsen, unpublished results). To our knowledge, however, it has not been detected outside this farm system. This is very fortunate since circulation of H4 viruses would represent an antigenic shift to a subtype against which pigs have no immunity. Likewise, this virus would also represent a dangerous antigenic shift for the human population. None of the farm personnel reported influenza-like illness at or immediately following the time of the swine disease outbreak, although serum samples could not be obtained from these individuals to assess subclinical infections. However, the HA of this virus contained amino acids in the receptor binding pocket that have been associated with  $\alpha$ 2,6 (human) receptor binding ( [Karasin; Connor; Ito; Naeve and Rogers](#)). Conversely, the PB2 of this virus maintained an avian signature, lacking mutations at amino acids 199, 661, 667 ( [Hiromoto et al., 2000](#)) and 627 ( [Subbarao et al., 1993](#)) that have been associated with a human host-range. Overall, the risk for human infection with this virus remains unclear.

## 7. Summary

The epidemiology of influenza among pigs in North America has changed dramatically since 1997. Viruses of H3N2, H1N2 and H4N6 subtypes have been isolated from pigs after a period of over 60 years in which swine influenza was caused almost exclusively by infection with H1N1 viruses. The H3N2 and H1N2 viruses appear to have become widely established within the swine population of the United States. Beyond traditional antigenic typing, extensive genetic analyses have been conducted in order to understand the evolutionary origin of these viruses. If we are to understand the on-going evolution of influenza viruses in pigs and detect atypical viruses in the swine population as a part of human pandemic preparedness, it is important that genetic characterization of swine isolates continues.

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Tel.: +1-608-265-8681; fax: +1-608-263-0438; email: [olsenc@svm.vetmed.wisc.edu](mailto:olsenc@svm.vetmed.wisc.edu)

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