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Epidemiology of swine influenza

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Introduction

For influenza viruses and their epidemiology, the only constant is change. Although this principle is perhaps best exemplified by human influenza viruses, the recent emergence of reassortant H3N2 influenza viruses among pigs in the United States reinforces the fact that pigs are by no means excluded from this pattern. In fact, a variety of unique swine influenza viruses have been isolated around the world in recent years. This paper will present these viruses as examples of how influenza viruses can change, and it will show the potential impact of influenza virus variation on the swine industry in the United States. In addition, it will review the results of recent studies on the nature of influenza viruses circulating among pigs in the United States from 1997 to the present.

Background

Influenza virus structure and ecology

Influenza A viruses (the most common type of influenza virus infecting animals, including people) contain 8 segments of RNA that encode 10 viral proteins. The two large surface proteins on the virus are the hemagglutinin (H or HA) and neuraminidase (N or NA). (See **Figure 1**.) The HA contains the important antigenic sites to which protective antibodies are directed, and antigenic and genetic differences in the HA and NA define an influenza virus's subtype, e.g., H1N1, H3N2, and so on.[34] In total, there are currently 15 HA and 9 NA subtypes recognized among influenza A viruses in pigs.[48]

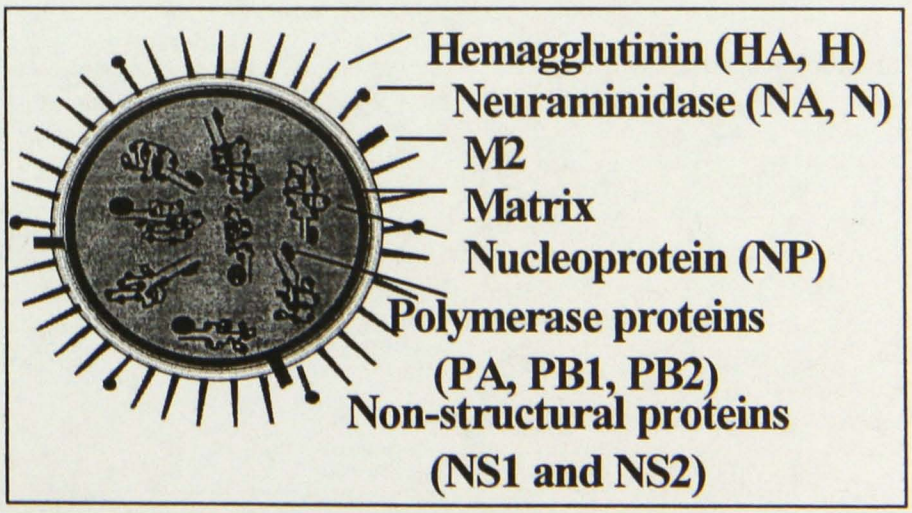
To understand the epidemiology of influenza in pigs, it is critical to realize that influenza viruses can infect a variety of other species; in fact, inter-species infection can be a mechanism for introduction of novel strains of influenza viruses into the swine population. Influenza viruses are important pathogens in a variety of domestic and non-domestic species,[34,63] including the following taxa:

- chickens
- turkeys
- horses
- seals and whales

Most importantly, however, influenza viruses also infect ducks and other waterfowl. In fact, all 15 HA and 9 NA subtypes of influenza exist among waterfowl. In these species, influenza viruses target the gastrointestinal tract rather than the respiratory tract, and the infections are completely subclinical. These attributes, along with the migratory behavior of waterfowl and the ability of

Figure 1

Influenza virus schematic



influenza viruses to persist in cold lake water, contribute to the capacity of waterfowl to form an immense reservoir for influenza viruses in nature.[63]

Influenza in pigs

Influenza viruses were first isolated from pigs in the United States in the early 1930s.[55] These were H1N1-subtype viruses, and their isolation from pigs followed closely upon the 1918 human H1N1 influenza pandemic that claimed over 20 million lives around the world.[34] In fact, influenza-like disease was recognized clinically among pigs in the United States during the late summer and fall of 1918,[18,28] and recent evidence clearly indicates that the 1918 human virus and the 1930s swine viruses were closely related.[45] However, it remains unclear whether these viruses appeared first in people and then spread to pigs, or vice versa.

H1N1 influenza viruses have continued to infect pigs in the United States ever since the 1930s, and influenza remains one of the most commonly encountered swine respiratory diseases. It can present as either an enzootic disease problem in a herd or, more commonly, as explosive outbreaks of acute respiratory disease with fever, anorexia and weight loss, lethargy, nasal and ocular discharge, coughing, and dyspnea.[18] The severity of clinical illness is dependent upon the following factors:

- The strain of influenza virus involved
- The age of the pig
- The immune status of the pig
- Environmental conditions
- The presence or absence of concomitant stress factors
- The presence or absence of additional respiratory tract bacterial and/or viral pathogens

Under these last conditions, there is particular concern for the impact of synergistic infections between influenza virus and porcine reproductive and respiratory syndrome virus or porcine respiratory coronavirus.[21,26,61,62]

Public health implications of swine influenza

Beyond the importance of influenza as a disease problem for the swine industry, pigs also play two important roles in the epidemiology and ecology of influenza viruses for humans. First of all, classical H1N1 swine influenza viruses are zoonotic pathogens. Human infections with swine influenza viruses have been documented in the US at least nine times since 1974,[14,23,65] including fatal infections,[27,43,47,56,58,64] as well as in Europe,[15] and in New Zealand.[17] In addition, data suggest that zoonotic swine influenza virus infections may actually occur more routinely among people in regular contact with pigs than the number of documented cases would suggest.[39,49]

Secondly, because pigs are susceptible to infection with both avian and mammalian influenza viruses (their tracheal epithelium contains receptors appropriate for viruses from both lineages[24]), they have been implicated as the intermediate host for adaptation of avian viruses to mammals[8] and as the "mixing vessels" in which human-avian virus reassortment occurs.[50,52,63] Reassortment is possible for influenza viruses because of the segmented nature of their RNA. When two different influenza viruses infect an individual simultaneously, the viruses can interchange their genes during replication and create an entirely new virus.[34] The major pandemics of human influenza this century (1957, 1968) were caused by novel virus strains that arose by reassortment between the pre-existing human influenza viruses of the time and avian influenza viruses.[63] Although the role of pigs in this process was only a theory for many years, human-avian influenza virus reassortants have been isolated recently from commercially-raised pigs in Europe[10] and subsequently from children in the Netherlands.[12] Finally, older strains of human influenza virus can be maintained among pigs,[1,25,32,35,41] thereby allowing for re-introduction of antigenic variants back into the human population.

Swine influenza virus epidemiology in the United States: 1976–1989

Surveillance studies conducted in the United States during 1976/1977[23] and 1988/1989[11] demonstrated that influenza virus exposure was consistently highest among pigs in the north-central states, with seropositivity rates against classical swine H1N1 viruses of 20–47% in 1976/1977 and 51% in 1988/1989. In contrast, serologic evidence of H3 influenza virus exposure was remarkably lower in both studies (1.4% in 1976/1977 and 1.1% in 1988/1989). In 1988/1989, sera were also tested for antibodies to an avian virus, A/Duck/Alberta/16/87, but none of the 2,337 samples tested contained detectable antibodies to this virus. These surveillance studies clearly demonstrate that H1 influenza viruses have been the predominant subtype circulating among pigs in the United States since 1976. Nonetheless, variant H1 viruses and other subtypes of influenza viruses have been isolated recently from pigs in North America, Europe, and Asia. The appearance of atypical strains can impact the design and use of swine influenza virus vaccines and potentially impact our ability to recognize and diagnose influenza. In addition, novel influenza viruses that appear in pigs may threaten human health. Examples of variant swine influenza viruses are presented below to illustrate the ways in which influenza viruses evolve and change.

Mechanisms of virus variation and examples of novel swine influenza viruses

New strains of influenza viruses in a given species of animal can be generated by one of the three methods:

- Antigenic drift
- Antigenic shift/virus reassortment
- Inter-species transmission

Antigenic drift

Antigenic drift is a process of subtle, gradual change in the antigenic make-up of a virus that occurs because of specific "point mutations" in the genes encoding the HA and, to a lesser extent, the NA. The force that drives antigenic drift is "immune pressure," i.e., the presence of virus neutralizing antibodies in an animal forces the virus to change so as to "escape" neutralization.[34] Antigenic drift occurs very commonly among human influenza viruses, and it is the reason that human influenza virus vaccines are updated with new strains every few years. In contrast, swine viruses have historically undergone comparatively less antigenic drift,[37,54] although two unique antigenic drift variants of H1N1 swine influenza viruses were isolated in 1991/1992 in North America.

A/Swine/Nebraska/1/92 (Sw/NEB)40

This H1N1 virus was isolated during a severe disease epizootic on a commercial swine farm in Nebraska. Clinical signs differed from those typically seen with swine flu in that the infected pigs had very high fevers for prolonged periods of time, yet showed relatively mild respiratory signs and very little coughing. Interestingly, however, when pigs were experimentally infected with Sw/NEB under controlled conditions, the clinical signs and pathologic lesions were similar to those seen following classical swine influenza virus infection. Therefore, the clinical presentation in the initial outbreak was probably partially dependent upon secondary pathogens. The HA of Sw/NEB is antigenically and genetically distinct from classical swine influenza strains, and it is suspected that management practices on the farm of origin allowed this virus to continually re-circulate, thus generating more intensive immune pressure for antigenic drift.

A/QC/5393/9116,33,46

In 1988, an unusual form of pneumonia was recognized among pigs in seven swine herds in Quebec. The pigs demonstrated fever and dyspnea, but once again coughing was not a consistent finding. Pathologically, these pigs had very unique lesions, and the lesions were reproduced in experimental infection studies. Grossly, the lungs from affected animals were very meaty and rubbery, and were described as looking more like thymus than pulmonary tissue. Histologically, there was a profound proliferation of type II pneumocytes, as well as an infiltration of mononuclear inflammatory cells. An H1N1 influenza virus was isolated from these pigs and, like Sw/NEB, genetic characterization suggested that this virus was also an antigenic drift variant of classical swine H1N1 influenza viruses.

Antigenic shift

Antigenic shift is a more dramatic form of virus variation that occurs when a virus with a completely different subtype of HA and/or NA appears in a population. These viruses develop as a result of genetic reassortment events. Examples of reassortant swine influenza viruses include the following:

H1N2 reassortant virus

H1N2 reassortant viruses were isolated from pigs in Japan in 1978[46] and 1989/1990,[42] France in 1987 and 1988,[20] and the United Kingdom in 1994.[4] These viruses were reassortants between human and swine viruses, although a multiply-reassorted H1N2 virus with human, swine, and avian genes has more recently been isolated in the United Kingdom.[6] Epidemiologically, the French and Japanese viruses did not spread extensively. However, the H1N2 viruses in the United Kingdom became widely established and have caused multiple outbreaks of respiratory disease in pigs.[2,4]

H1N7 reassortant virus

In 1992, an H1N7 reassortant virus was isolated in the United Kingdom. This virus was a reassortant between human (HA) and equine (NA) influenza viruses,[3] and it is of particular significance because equine H7N7 viruses have not been isolated from horses anywhere in the world since 1980. The clinical presentation associated with this H1N7 virus on the farm of origin was typical for swine influenza, but experimentally the virus induced only very mild signs and replicated to such a limited extent that only one inoculated pig seroconverted.

Although these viruses both maintained the H1 subtype characteristic of pre-existing swine influenza viruses, future reassortant viruses could pose a substantial risk to the population if a shift occurs in HA subtype. This is because individuals will not have any pre-existing immunity to the new HA protein.

Inter-species transmission

Inter-species transmission of entire influenza viruses (i.e., without reassortment) can clearly occur, e.g., zoonotic swine influenza virus transmission to humans. However, pigs can also be the recipient host of influenza viruses from either humans or birds. In the United States, there has historically been very little evidence for human or avian influenza virus infections in pigs. However, this has not been the case elsewhere in the world.

Human H3N2 virus

Human H3N2 viruses have been regularly isolated from pigs in Europe and Asia.[8,9,32,35,41] These infections generally induced similar or less severe clinical disease than infection with the traditional H1N1 swine influenza viruses.[18,51]

Avian H1N1 virus

Avian H1N1 viruses have posed substantial problems for the swine industry in Europe. Sometime prior to 1979, an avian H1N1 virus crossed the species barrier to pigs. Since then, it has become the dominant swine H1N1 virus subtype throughout northern Europe[44,63] and has caused extensive outbreaks of influenza in the European swine population. In 1992, a similar avian H1N1 virus was isolated in England,[5,7] and avian H1N1 viruses have also been isolated from pigs in Southeast Asia[22] and Ireland.[30]

Avian H5N1 virus

An avian H5N1 virus crossed the species barrier from chickens to people (most likely via contact in live-bird markets) in Hong Kong in 1997.[13,19,57,66] This was a dramatic and frightening event because 6 of the 18 people that were infected died. This was the first time that an entirely avian virus infected and caused significant disease in people. Although it would be reasonable to consider that the virus initially infected pigs as an intermediary mammalian adaptation host, this does not appear to have been the case. There are no published accounts of infection of pigs with this virus.

Swine influenza virus epidemiology in the United States since 1998

The examples outlined above clearly indicate that a variety of antigenic drift and shift variants of influenza virus, as well as human H3N2 and avian H1N1 viruses, have been isolated from pigs around the world in recent years. In light of these changes, and since a major survey of swine influenza viruses in the United States had not been conducted since 1988/1989, we felt it was important to re-examine the nature of influenza virus among American pigs. Therefore, from September, 1997 through August, 1998, we undertook a virologic and serologic evaluation of influenza virus infections among pigs in the north-central United States, principally Wisconsin and Iowa.[38] We specifically wanted to address two questions.

- Are antigenic variants of swine H1N1 influenza viruses circulating among pigs in the United States?
- Are pigs in the United States being exposed to human H3 influenza viruses to a greater degree than in the past, as well as avian H1 viruses?

A total of 2,375 serum samples were obtained from two sources. A subset of 1,175 samples were selected randomly (approximately 100 samples/month) from sera submitted to the Wisconsin Animal Health Laboratory for pseudorabies virus testing, and another 1,250 samples (50 samples approximately every 2 weeks) were collected from pigs at the time of slaughter in a commercial abattoir. Samples of nasal secretions were also collected for virus isolation from the slaughter pigs. (The abattoir received pigs principally from southwest Wisconsin and northeast Iowa, and, to a lesser degree, from northwest Illinois.) Serum samples were tested by hemagglutination-inhibition (HI) assay against four reference influenza viruses:

- A/Swine/Indiana/1726/88 (Sw/IND), a classical swine H1N1 virus
- A/Bayern/7/95 (A/BAY), a H1N1 human influenza viruses representative of the viruses circulating in the US during the two years prior to our study
- A/Wuhan/359/95 (A/WUH), a H3N2 human influenza viruses representative of the viruses circulating in the US during the two years prior to our study
- A/Duck/Alberta/35/76 (Dk/ALB), a well-characterized avian H1N1 virus

Nasal swab samples were inoculated into embryonated chicken eggs to allow for growth and isolation of influenza viruses.

A total of 26 influenza viruses were isolated during the course of the study, but a distinct seasonal pattern was observed. Twenty-four of 26 were obtained from October through January, yielding a peak virus recovery rate of 16% during that time. Genetic analyses of the HA and NP genes of 11 representative isolates (pairwise comparisons to prototypical swine, human, and avian viruses, as well as phylogenetic estimations of evolutionarily relatedness) demonstrated that the viruses were derived from the classical swine H1 virus lineage. Despite this genetic conservation, however, substantial antigenic variation among the viruses was detected. Hemagglutination-inhibition assays, using a panel of four monoclonal antibodies previously shown[31,54] to recognize distinct epitopes in three antigenic sites on the H1 HA, revealed eight different reactivity patterns. In fact, none of the viruses were antigenically identical to our reference swine H1 virus, Sw/IND. Of practical significance, however, all of the viruses reacted to the same titer as Sw/IND with sera from experimental pigs that had received two doses of MaxiVac-Flu(r) (Syntrovet) vaccine. Therefore, the level of antigenic drift currently present among swine H1 influenza viruses in the north-central United States is unlikely to impact the efficacy of the currently-available vaccine. However, in order to make rational decisions regarding vaccine strain selection in the future, it will be

important to continue to monitor the antigenic make-up of contemporary swine influenza viruses.

Several aspects of our serologic findings deserve comment. First, 27.7% of pigs were seropositive (HI titer 1:40) to Sw/IND. It is likely that the vast majority of these seropositive pigs had virus-specific antibodies because of previous infection rather than vaccination. Approximately nine million doses of MaxiVac-Flu vaccine were sold nationwide in 1998 (J. McMillen, personal communication). However, only about 40% of the vaccine was used in slaughter pigs, the remaining being used in breeding animals (R. Sibbel, personal communication). Therefore, since 92 million pigs went to slaughter in the United States in 1997[59] and 101 million in 1998,[60] and if animals received the recommended two doses/animal, and if we assume relatively uniform usage throughout the country, then only 1.8–2.0% of slaughtered animals would be expected to be seropositive because of vaccination. As such, a 27.7% seropositivity rate suggests that H1 influenza viruses continue to circulate widely within the pig population of the north-central United States.

Rates of seropositivity to human and avian H1 influenza viruses were lower, specifically 3.3% to human H1 (A/BAY) and 7.6% to avian H1 (Dk/ALB). However, these rates are significant because they indicate that human H1 influenza viruses at least occasionally infect pigs as a reverse zoonosis and that pigs in the United States are also being exposed to avian influenza viruses. While it is possible that these seropositivity results were due to cross-reactivity following infection with swine H1 virus, results of another experiment indicate that this is unlikely. Specifically, sera from pigs experimentally infected with Sw/IND,[29] and having an HI titer of 1:256 to Sw/IND, did not contain any detectable antibodies to A/BAY, Dk/ALB or A/WUH.

Perhaps the most interesting finding from this study was that 8.0% of the pigs tested positive serologically to human H3 influenza virus. Although H3 viruses have been routinely detected among pigs in Asia and Europe,[8,9,32,35,41] infection of pigs in the United States with this subtype has been rare in the past.[11,23] However, in August, 1998, an H3N2 influenza virus was isolated from a pig during a severe disease outbreak on a farm in North Carolina.[67] Additional H3N2 viruses were obtained from pigs in Texas, Minnesota, and Iowa in November and December, 1998,[67] and from pigs in Iowa, Nebraska, and Minnesota in March, 1999.[53] Genetic characterization of the 1998 NC, MN, and IA isolates indicated that the HA and NA genes from each of these viruses are of human influenza virus origin, while the internal protein genes are either all of swine virus origin or a mixture of swine and avian virus origin.[67] Likewise, preliminary results indicate that the internal NP genes of the 1999 isolates are most closely related to those of swine viruses, while the HA genes are from the human influenza virus lineage, and specifically more closely related to the older A/Wuhan/359/95 strain than to the more recent A/Sydney/5/97 strain.[53]

Conclusions

Atypical influenza viruses have been isolated from swine in the US and around the world over the last two decades. In some cases (e.g., the unusual H1N7 reassortant virus), these were "oddities" of virology that posed little threat to the swine industry. In other cases, however, the viruses (e.g., the H1N2 viruses in the United Kingdom) became important pathogens in the regional swine industry.

The current level of antigenic drift among the classical swine H1 viruses in the United States does not appear to be sufficient to require a change in H1 vaccine strain. However, the degree of antigenic variation in the swine H1 viruses must be continually monitored if we are to stay abreast of significant changes in the future.

Introductions of human and avian influenza viruses into the United States swine population pose a risk because of the potential for the appearance of subtypes of virus to which our pigs have little or no immunity. In this regard, the emergence in the last year of H3N2 viruses among American pigs presents a real concern for widespread influenza epizootics in pigs. These viruses also pose new challenges for the swine industry and the veterinary community. Specifically, we need to develop enhanced biosecurity approaches to prevent the introduction of human and avian influenza viruses to pigs in the future. We must also develop an effective bivalent H1 and H3 influenza virus vaccine to stem the tide of the current H3 swine influenza epizootic.

Our serologic results support the fact that pigs in the north-central United States were being exposed to both human H3 viruses and avian H1 viruses for at least one year prior to the first H3N2 isolate from a pig in North Carolina. As such, the necessary ingredients were present to generate these H3N2 reassortant viruses, and the potential exists for the emergence of additional swine/human/avian virus reassortants among pigs in the United States in the future. Furthermore, although the 1998/1999 H3N2 reassortants contained HA and NA subtypes to which people have already been exposed, there is no guarantee that this will be the case the next time. Generation of a reassortant with a non-H1 or H3 avian HA would pose significant risks for both pigs and people. Therefore, continual aggressive surveillance of swine influenza viruses should be included as part of an overall approach to the prevention of influenza epizootics in pigs and human influenza pandemics.

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