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Epidemiology of swine influenza and implications of reassortment

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Swine influenza virus was first isolated by Dr. Richard E. Shope in 1930 (19). This original H1N1 SIV was considered the primary ancestor of the H1N1 SIVs circulating today in North American pigs and is often referred to as "classical" H1N1 SIV (27). SIV has since evolved from this classical H1N1 and changed through viral genetic means to become a complex collection of viruses undergoing continuous change. These viral changes are some of the most important factors affecting the epidemiology of swine influenza throughout the world (11, 34).

Influenza viruses accumulate changes by two mechanisms: antigenic drift and antigenic shift/reassortment (20). Antigenic drift describes the process by which influenza A genes HA and NA acquire nucleotide point mutations, substitutions, deletions, and insertions that may result in amino acid translation differences in the antigenic sites of the molecules (8). Reassortment is the exchange of genes between two or more influenza viruses with the production of a new variant or reassortant viruses (4, 25). Specifically, HA and NA reassortments are termed "antigenic shifts" because the HA or NA gene segments between two or more influenza viruses infecting the same host cell are swapped, resulting in completely new subtypes (e.g., H1N1 reassorting with H3N2 to produce H1N2) that can have dramatic effects on the population infected (20, 22). All these processes have been involved in creating the SIV situation that exists today.

From the time of its recognition in 1930 until 1998, classical swine H1N1 was the predominant SIV in North America. Classical swine H1N1 was shown to exhibit some antigenic changes due to drift but, all in all, the virus was considered a stable genotype in the United States (26). In 1998, H3N2 SIV emerged via reassortment events causing widespread respiratory disease across the United States (37). The first H3N2 SIV to appear was in pigs in North Carolina (A/Sw/NC/98). A/Sw/NC/98 was found to possess human HA, NA, and PB1 genes most closely related to human H3N2 viruses circulating in 1995 as well as swine PB2, PA, NP, M, and NS genes of classical swine H1N1 viruses, making it a double reassortant (38). The next H3N2 viruses to appear almost simultaneously throughout the United States were triple reassortants of swine, human, and avian viruses. These triple reassortants possess swine NS, NP, and M genes; human HA, NA, and PB1 genes; and avian PB2 and PA genes. These triple reassortants fall into three groups or "clusters" based upon analyses of their HA gene sequences (35). Cluster I includes A/Sw/TX/98 that appears to be most closely related to H3N2 viruses isolated from humans in 1995. Cluster II includes A/Sw/CO/99, which is closely related to the 1997/1998 season predominant human virus A/Sydney/97. The third cluster includes A/Sw/OK/99 and A/Sw/IL/99, viruses that are most similar to the predominant human strains of 1996—A/Wuhan/95-like H3N2 viruses (35). The clusters have both genetic and antigenic differences that further support their different ancestries (30). Prior to the infection of U.S. pigs with the double and triple reassortant H3N2 viruses, the only other H3N2 infections of pigs in North America in the 1990s involved wholly human H3N2 viruses. These infections in Canada occurred in Ontario in 1997 and in Quebec in 1991 and likely were reverse zoonoses that occurred sometime prior to their detection in pigs (21, 3, 35).

In early 1999, the HA of the pre-existing classical H1N1 SIV reassorted with H3N2 SIV virus to create a second reassortant virus, H1N2 (16). The H1N2 viruses were isolated from pigs in Indiana that were exhibiting respiratory disease, fevers, and inappetance (16). Genetic analyses performed at the University of Wisconsin revealed HA gene similarities to swine H1N1 viruses of the classic H1N1 origin, but the remainder of the genes were very similar to the recently emerged H3N2 SIV isolates (17). H1N2 SIV persists in U.S. swine and was found in pigs from at least 9 states in a retrospective study of H1N2 isolates recovered from 1998 to 2001 (6).

Reassortment events continued. In 2000, seemingly novel reassortant H1N1 SIV viruses were detected that possessed HA and NA genes from classical swine H1N1 viruses and the six internal or structural genes of H3N2 SIV (33). Reassortant H1N1 SIV not only possesses the internal genes of H3N2 SIV, specifically the avian-like PA and PB2 polymerases, but also there have HA genetic and antigenic changes that are capable of escaping neutralization by anti-H1 antibodies in immunized pigs (33). Acquisition of avian-like polymerases is also significant because there appears to be a selective advantage to these influenza viruses and they soon predominate in the population in which they have been established (35). Analyses

of archived viruses from 1997 and earlier have revealed the presence of avian PA and PB2 genes in H1N1 swine influenza viruses, suggesting that the reassortment may have occurred prior to 2000 and was undetected (36).

Reassortment between avian, human, and swine influenza viruses is expected to occur in pigs owing to several epidemiological factors:

- Pigs have the appropriate cellular receptors to receive both avian and human influenza viruses (15);
- Pigs have been implicated as the intermediate host for mixing viruses in past epidemics (1, 29);
- There is a continual population of susceptible swine born daily that have no immunity to influenza (5, 10); and
- Herds of swine exist that are in close association with human and bird populations (14).

These recent influenza A viruses have complicated disease management and control. This is due in large part to endemic SIV continually infecting susceptible pigs (4), some of which are subclinically infected but shedding virus, and the presence of several SIV strains co-circulating in a herd at the same time (7, 34). Some swine herds experience severe influenza-induced respiratory disease despite vaccination, the ramifications of which are poor performance in growing swine and herd loss. These continual losses are frustrating to producers and veterinarians.

In an attempt to determine the implications these reassortant SIV have on swine health and production, antigenic and genetic characterizations have been performed by the Minnesota Veterinary Diagnostic Laboratory (MVDL) on numerous virus isolates. Antigenic characterizations conducted via hemagglutination inhibition tests performed using a panel of antisera for several H1 and H3 viruses revealed antigenic diversity among both H1 and H3 viruses. The reassortant H1N1 viruses recently isolated from swine were inhibited by classical H1N1 antisera to a much lesser extent than was found in homologous HI reactions. Furthermore, detection of convalescent antibodies in sera from swine infected naturally with reassortant H1N1 viruses was inconsistent or absent especially when compared to seroconversion to the homologous infecting field strain of reassortant H1N1 virus. H3 viruses were increasingly non-reactive to reference A/Sw/TX/98 antisera. Overall, the percent of SIV isolates that were non-typable by HI serotyping with reference classical H1N1 or A/Sw/TX/98 antisera increased from 2001 (11%) to 2003 (13%).

Genetic characterizations of both H1 and H3 influenza viruses performed at the MVDL by comparing sequences of the HA gene has revealed several groups of divergent virus variants. In an analysis of 155 H1 field viruses, three phylogenetic groups were apparent: a reassortant H1N1-like group that included approximately 50% of the viruses sequenced, an H1N2-like group (35%), and a classical H1N1-like group (15%). The H1 sequence information did not predict NA type, nor did it reveal the presence of avian internal genes. Within groups, there was a maximum of 5% nucleotide difference and between groups the nucleotide differences were as high as 12%. H3 virus phylogenetic analyses were consistent with previous analyses and demonstrated the three clusters of H3 viruses. An analysis of 88 field viruses revealed that the majority of H3N2 viruses sequenced by the MVDL in 2003 were A/Sw/IL/99- and A/Sw/OK/99-like (cluster III). Within a group, the nucleotide differences ranged from 0–8%.

Thus, antigenic and genetic variation clearly exists in the contemporary H1 and H3 SIV isolates, but the implication these variations have for vaccination and control programs is not evident without performing vaccination and challenge studies. While several vaccination and challenge studies have been performed in Europe with meticulously characterized isolates and inclusion of detailed measurements of disease development and viral shedding, few such experiments have been completed and published by independent researchers in the U.S.

In a study in Belgium, pigs were vaccinated with three different killed H1N1 SIV variants or with a bivalent H3N2/H1N1 commercially available vaccine. Pigs were then experimentally inoculated two weeks after the final vaccination with a contemporary H1N1 virus known to be circulating in the Belgian swine herds. They concluded that vaccination with homologous or a similar current strain of H1N1 induced high post-vaccination antibodies, reduced clinical signs and decreased viral shedding when compared to the commercially available vaccine derived from a historical strain (32). They reported a statistical difference in the mean post-vaccination HI antibody titers against the eventual challenge virus strain. The geometric mean titers were 1:91 and 1:97 for the contemporary strains vaccines versus a titer of only 1:16 for the pigs vaccinated with the historical H1N1 strain. However, there was an equally high geometric mean titer of 197 present against the challenge virus for the pigs vaccinated with the commercially available vaccine (32). Thus, protection against variant viruses of H1N1 SIV was possible even when using a vaccine strain that was genetically and antigenically different from the challenge strain if the vaccination-induced antibodies were sufficiently high (32).

Studies on the efficacy of H3N2 vaccine have been published in peer-reviewed journals in Canada (3) and in the Netherlands (13). The Canadian work was a response to the emergence of H3N2 influenza A virus in Canada in 1991 that was reported to cause a proliferative pneumonia in swine different from the described influenza-asso-

ciated histopathological lesions (3). The clinical signs and magnitude of viral shedding were studied for the immediate four days post-challenge with a field isolate of the recently discovered H3N2 in pigs previously vaccinated with a killed vaccine generated from the same field isolate (3). Again, high HI antibody titers were produced (geometric mean titer=1:60) following vaccination. After homologous challenge, there was prevention of all clinical signs and no detectable virus produced in vaccinated-challenged pigs. In contrast, non-vaccinated but challenged pigs experienced fevers, tachypnea, with the majority of pigs shedding virus from 1–4 days post-inoculation (3).

The study in the Netherlands regarding H3N2 vaccination and challenge (13) was in response to work regarding the antigenic and genetic differences in current field isolates of SIV H3N2 in Europe. These strains demonstrated lack of cross-reactivity in HI tests (9). Thus, Heinen and colleagues set out to supplement the in vitro work of de Jong by performing in vivo studies. Heinen inoculated pigs with a contemporary 1998 H3N2 field isolate to induce a homologous immunity. Another group of pigs was vaccinated per label instructions with the commercially available H3N2 vaccine derived from an H3N2 first isolated in 1973. Both groups were challenged several weeks later with the contemporary H3N2 field isolate. The parameters for assessment of protection were clinical signs of disease, duration and magnitude of viral shedding, and transmission of the virus to in-contact, naïve pigs. While both the vaccinated and the homologous inoculated pigs had no clinical signs and did not transmit virus to the in-contact pigs, the vaccinated pigs did have detectable virus shedding in the lung tissue and in oropharyngeal swabs at days 1 and 2 post-challenge. However, the shedding was at very low levels <1.60 $\log_{10} TCID_{50}$/ml in the vaccinated group compared to approximately 3.7 $\log_{10} TCID_{50}$/ml for the non-immunized pigs (13). The conclusions of this study were that lack of cross-reactivity in vitro does not necessarily correlate with lack of cross-protection in vivo. Furthermore, they noted that homologous protection was superior to heterologous protection. However, heterologous protection was still efficacious in that it prevented transmission of virus to naïve pigs and prevented overt clinical disease that may dampen pig performance. Finally, they suggest that immunity and protection of the pig from SIV infection likely relies heavily on mucosal and cellular immune responses that need to be further elucidated (13).

The majority of vaccination and challenge studies in the U.S. are conducted by vaccine manufacturers. The goal of most of the studies was to demonstrate strong antibody response, reduction of clinical signs, lesions, and viral shedding in bivalently vaccinated pigs, experimentally inoculated with homologous or heterologous strains of field SIV virus (12, 24, 18, 31, 28). The results of these studies were usually favorable.

Vaccination and challenge studies to evaluate the protection of Pfizer FluSure(tm) H1N1/H3N2 bivalent vaccine against reassortant H1N1 or H3N2 viruses were performed at the MVDL as extensions of diagnostic investigations sponsored by Pfizer Animal Health. Groups of three-week-old, SIV, porcine reproductive and respiratory syndrome virus, and *Mycoplasma hyopneumoniae* seronegative pigs were either vaccinated per label directions with FluSure(tm) or non-vaccinated. Pigs were then intranasally challenged two weeks post 2nd vaccination with a reassortant H1N1 virus—A/Sw/MN/02—a genetically and antigenically different virus. A third group of pigs served as negative controls. For one-week post-challenge, pigs were observed for clinical signs, nasal swabs were taken and rectal temperatures were recorded. All pigs were then euthanized and complete necropsies performed on day 7 post-challenge. A similar study using an A/Sw/IL/99-H3N2-like virus is in progress.

The results were favorable. The challenge virus used had genetic differences from the Pfizer H1N1 vaccine virus (91.9% HA gene nucleotide similarity and 90.2% amino acid similarity). Antigenic differences were apparent as well, as previously collected convalescent sera from pigs naturally infected with A/Sw/MN/02-like viruses had poor seroconversion to classical H1N1 or Pfizer H1N1 vaccine virus. In this study, all vaccinated pigs demonstrated a high antibody titer response (GMT 1:381) to vaccine, with some cross-reaction (GMT 1:80) against the challenge virus. The titers remained high against vaccine and challenge virus throughout the observation period. There was low titer response detected by classical H1 HI or SIV Idexx H1N1 ELISA tests. Non-vaccinated pigs had no antibodies prior to or at the time of challenge. An antibody titer response to challenge virus at 7 days post-challenge indicated successful infection. Again, no antibodies were detected by ELISA. The vaccinated pigs had reduced viral shedding with only one pig virus isolation positive on a nasal swab collected at 3 days post-challenge. All non-vaccinated pigs were virus isolation positive on days 1, 3, and 5 days post-challenge. Negative control pigs were virus isolation negative at all time points. Clinical signs of disease were mild in both the vaccinated and non-vaccinated groups, but more pigs had nasal discharge or were lethargic in the non-vaccinated group compared to the vaccinated group. Gross pneumonia was minimal or absent in all vaccinated pigs (average gross lung pneumonia score 0.1%) and miminally present (average gross lung pneumonia score 2.53%) in the non-vaccinated pigs.

In this H1N1 vaccination and challenge study, vaccination with Pfizer FluSure™ H1N1/H3N2 reduced viral shedding, clinical signs, and pneumonia when pigs were

infected with a heterologous reassortant H1N1 virus. Similar results are expected from the H3N2 vaccination and challenge study and such favorable results have been reported by several vaccine manufacturers when their vaccines are used in controlled studies.

Why, then, are there so many anecdotal reports of apparent vaccination failure? Perhaps it is, as researchers suggest, that efficacy of the vaccine used likely has as much to do with virus selection as adjuvant, timing, and presence of maternal antibody interference (23). Diligent disease monitoring, serological, and viral surveillance are keys to understanding the dynamics of swine influenza in all herds. Controlled experiments coupled with detailed diagnostic investigations should prove to be beneficial for producers, veterinarians, vaccine manufacturers, public health professionals, and researchers all trying to understand the epidemiology of swine influenza and this continually changing and elusive virus.

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