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# Emergence of reassortant influenza viruses: Academic folly or clinically relevant?

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Influenza virus is an important cause of respiratory disease in swine. Of equal importance is its role as a zoonotic pathogen, capable of infecting swine, humans, birds, horses, dogs, cats and marine mammals. In 1997, focus on influenza viruses intensified due to avian influenza virus infections and deaths in humans in Asia. Since then, there has been a worldwide plea to examine all influenza viruses more thoroughly, in the hopes of heading off an influenza pandemic. Since 2001, the Minnesota Veterinary Diagnostic Laboratory, with the support of Pfizer Animal Health and in collaboration with several laboratories and pharmaceutical companies, has been genetically characterizing influenza viruses isolated from swine. What's been discovered are several variants of swine influenza viruses that, through their ability to continually change through reassortment and genetic drift, continue to be challenging respiratory pathogens.

Influenza viruses belong to the family Orthomyxoviridae. They are segmented, negative-sense, RNA viruses containing 8 RNA segments that code for at least 10 viral proteins. There are three types of influenza viruses – type A, type B, and type C. Influenza type is determined by the genetic characteristics of the conserved RNA genes, nucleoprotein (NP) and matrix (M). All swine influenza viruses in North America are type A unless proven otherwise. Type A influenza viruses are further subtyped by their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), encoded for by their RNA segments of the same name. There are 16 hemagglutinin subtypes, H1 – H16, and 9 neuraminidase subtypes, N1 – N9. From 1930 to approximately 1998, swine were infected with only one subtype – H1N1. This "classical" H1N1 appeared relatively stable for over 60 years. In 1998, H3N2 viruses emerged in swine. The majority of these H3N2 viruses were triple reassortant viruses, containing gene segments from human, avian, and swine influenza viruses. Since 1998, several additional reassortments have occurred. In 1999, H1N2 viruses emerged from a reassortment event between classical H1N1s and triple reassortant H3N2s. "Variant" H1N1 viruses containing avian internal (polymerase) genes were first detected in 2001 but were probably present much earlier. When "variant" H1N1 viruses emerged, they were found to have HA genetic and antigenic differences, so much so that well vaccinated adult swine with demonstrable H1N1 swine influenza hemagglutination inhibition (HI) antibody titers developed clinical influenza when infected with a variant H1N1 (A/Sw/MN/2002). What soon transpired was a platform of viruses capable of co-infecting herds, reassorting, and undergoing more rapid antigenic drift (e.g., subtle changes resulting from mutations in the genetic sequence of the HA gene), and antigenic shift (e.g., dramatic changes resulting from reassortment or swapping of virus gene segments between viruses).

The significance of a platform of H1N1, H1N2, H3N2 and variant H1N1 viruses infecting US swine is being elucidated through collaborative research efforts between producers, practitioners, diagnosticians, and researchers. We know that escape from vaccinal immunity can occur if the HA of the infecting strains varies antigenically and genetically from the HA of the vaccine strain. The exact amount or type of variation necessary to cause vaccine escape is not known, and detailed analyses of HA gene sequences and antigenic characteristics are necessary to determine what is a "significant" change and what is merely drift. The influenza viruses that tend to predominate in swine are those that have acquired avian genes, particularly the avian polymerase (internal) genes responsible for virus replication. This has proven true in the UK, EU and now in the US where our triple reassortant H3N2 viruses containing gene segments from avian, swine and human influenza viruses have displaced the double reassortant SIV containing only swine and human influenza virus gene segments. Also in the US, H1 swine influenza viruses that contain avian-like influenza virus internal genes have displaced the "classical" H1 swine influenza viruses comprised of all swine-like influenza viral genes.

Currently, nearly all swine influenza viruses contain these avian-like internal genes, and there appears to be two predominant H1 genotypes and one predominant H3 genotype. H1 viruses with an HA genotype similar to that of variant H1N1 viruses containing avian internal genes (e.g. A/Sw/MN/02) have replaced the classical H1 virus genotypes and predominate in Minnesota and Iowa. In other regions, especially the Southeast, while viruses of the variant H1N1 HA genotype are present in large numbers, the more H1N2-like or classical H1N1-like viral genotypes of H1 viruses predominate. These H1N2-like geno-

types are more similar genetically to classical H1N1 viruses than the variant H1N1 viruses, yet they are distinct enough from both to be considered their own genotype. The H3N2 viruses that predominate throughout the United States are most similar in HA genotype to the "Group III"-like H3N2 viruses such as A/Sw/IL/99 – H3N2. A/Sw/IL/99 – H3N2 viruses of swine are triple reassortants, but their HA gene is more similar to that of human H3N2 viruses that were circulating in 1996. The "Group III" or A/Sw/IL/99-like viruses have both genetic and antigenic differences from the "Group I" or A/Sw/TX/98-like viruses. Furthermore, the contemporary H3N2s circulating in US swine are drifting from A/Sw/IL/99-like viruses at a steady rate, so much so that to refer to them as Group III-like or A/Sw/IL/99-like is somewhat a misnomer.

While the vast majority of viruses isolated from swine in the US are genetically similar in the HA gene to one of the three predominant SIV subtypes mentioned above, occasionally new reassortants and variants emerge and infect pigs. In 2003 in the Midwest and in 2005 in the Southeast, several cases of respiratory disease in swine were caused by an influenza virus containing a human-like H1 gene similar to that circulating in humans since 1999. While reports of human illness on or near the swine farms were often not accompanying the swine respiratory cases, the importance of influenza as a zoonotic disease, or more accurately, an amphixenotic disease (transmitted in either direction) is reaffirmed by finding these human/swine reassortant influenza viruses. Also detected in 2004, a new subtype of influenza virus in swine was detected – H3N1. The H3 genotype is similar to that of contemporary swine Group III-like H3N2s and the N1 genotype is similar to that of classical H1N1. The clinical implications of H3N1 influenza on swine respiratory health is not known but is expected to be similar to that of H3N2 because the majority of the swine immune response to influenza is directed against HA protein. Of interest is the finding of H3N1 so long after the identification of both H3N2 viruses and H1N1 viruses co-circulating in US swine. Perhaps certain viral reassortment events are not as successful as others in creating viruses that will become well adapted to their host.

These reassortant and variant viruses are not only co-circulate in US swine, but they are also capable of greater rates of antigenic drift that result in changes to the HA protein. The changed HA is no longer completely recognized by the immune system, and outbreaks of swine influenza can occur in previously exposed (naturally or vaccinated) herds. The instability of the virus makes it a moving target, requiring diagnostic laboratories to update their serological test antigens to more closely match contemporary strains or use the actual infecting contemporary strains in the serological tests to measure immunity to the homologous virus. Trying to detect viruses through molecular methods is challenging if the primers used to amplify influenza genes are not targeted at regions that were previously regarded as conserved are now exhibiting variability. While the significance of some of the more "novel" reassortant viruses is not immediately clear, we will continue to address their emergence and examine their potential implications on swine health and vaccination policies.