# Clinical and Epidemiological Impact of the New Swine Influenza Strains: an American and European Perspective

Vicki J. Rapp-Gabrielson, PhD, and Robyn Fleck, DVM
Schering-Plough Animal Health Corporation (SPAH) Elkhorn, NE 68022, USA

## Introduction

Swine influenza is an important re-emerging disease, and the evolution and epidemiology of swine influenza virus (SIV) have been the subjects of recent review articles (1-4).  First recognized clinically in pigs in the United States in 1918, and first isolated in 1930, SIV is a highly contagious common respiratory pathogen of swine worldwide.  In naïve animals, the virus produces acute outbreaks characterized by high fever, nasal discharge, lethargy, cough and dyspnea.  Although usually of short duration, the high fevers can reduce feed intake and increase days to market.  In sow and gilts, high fevers may also result in abortion. In addition to the acute clinical signs, SIV infection can stress the respiratory tract to the point where secondary bacterial infections may become established.  Thus, SIV, along with *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome (PRRS) virus, is a major contributor to the porcine respiratory disease complex (PRDC).

Swine influenza virus belongs to the *Orthomyxoviridae* family of enveloped negative-stranded RNA type A influenza viruses that infect a large number of mammals. Aquatic birds are the primary reservoir of influenza A viruses and serve as asymptomatic carriers.  Swine are an epidemiologically important host because they can be infected with both avian and mammalian influenza viruses, and thus serve as a "mixing vessel" for the possible emergence of new viruses.  The genome of influenza A viruses consists of eight separate single-stranded segments of RNA that encode for ten structural proteins.  The two major envelope glycoproteins, the hemagglutinin (HA) and neuraminidase (NA), are antigenically important determinants that define the subtype of the virus.  The HA is involved with attachment of the virus to the host cells while the NA may be involved with release of the progeny virus from the infected cells. In addition to the HA and NA, influenza viruses contain three other major structural proteins: the nucleoprotein (NP) and the M1 and M2 matrix proteins.  The M and NP are the type-specific proteins that distinguish the influenza A, B, and C viruses. The remaining virus proteins are internal, consisting of three polymerases (PA, PB1 and PB2) that mediate RNA synthesis and two nonstructural proteins (NS1 and NS2) that are also involved with virus replication.

Although there are 15 different subtypes of HA and 9 different subtypes of NA, only three subtypes are of clinical importance in swine: H1N1, H1N2, and H3N2.  Within these subtypes, different lineages exist due to major genetic and antigenic differences the result of two major forms of evolution: antigen drift and antigenic shift.  The HA of the influenza virus is an important target for the host's immunological defenses. In order to evade the host's immune system, the virus has developed the ability to undergo continual structural change in the HA and NA glycoproteins. This change is called antigenic drift, and is a result of an accumulation of mutations that occur during replication of the virus. The gradual accumulation of these mutations result in antigenic changes, particularly to the HA, thus rendering the virus more likely to escape from recognition by the host's immune defenses.  In the past, it was thought that antigenic drift of the HA occurred more rapidly in human-adapted H1N1 viruses than in swine-adapted viruses because of the constant availability of a new population of naïve pigs available for the virus to infect.  However, in recent years, antigenic shift has been shown to be an important factor in the epidemiology of swine influenza infections.

The second major form of evolution, antigenic shift, involves the sudden emergence of new influenza strains different from those that had previously circulated in a population.  Antigenic shift may occur by direct transfer of a virus from a different animal species (i.e. avian to human) or by genetic reassortment between two or more viruses infecting the same host cell.  The result can be significant changes in the antigenic nature of the virus, resulting in

unrestricted spread throughout a host population.  In humans, influenza is a global disease and the major human influenza pandemics have been the result of antigenic shift.  In contrast, because swine populations tend to be more geographically restricted, the predominant subtypes and lineages differ between the North American and European continents.

## Epidemiology of North American SIV

Until 1998, influenza viruses North American pigs had remained genetically and antigenically stable for nearly 60 years. The only clinically significant virus circulating was the classical swine H1N1 (cH1N1) virus related to the virus involved in the human pandemic that occurred in 1918.  Evaluation of North American H1N1 viruses isolated as recently as 1997-98 indicated that while some genetic variability had occurred within the HA gene, there was still considerable cross-reactivity in the hemagglutination inhibition (HI) assay (5).  However, in 1998, the situation in the United States changed dramatically with the introduction of the triple reassortant H3N2 viruses (6).  These viruses have HA, NA, and PB1 genes derived from human H3N2 viruses, internal polymerase PA and PB2 genes derived from birds (likely an H9N2 virus) and the remaining internal genes from the classical swine H1N1 viruses (Table 1).  The triple reassortant viruses have become well established in US swineherds, with concomitant clinical outbreaks in naïve pigs during the winters of 1998, 1999 and 2000.  Newer H3N2 viruses have apparently derived their HA genes from different lineages of human H3N2 viruses by additional reassortant events, rather than antigenic drift.  The swine triple reassortant viruses have been classified into three "clusters" (7).  Cluster I contains the  "Texas 98" swine virus, with an HA most closely related to the human Wuhan 1995 strain.  Cluster II contains the  "Colorado 99" swine virus, with an HA most closely resembling the human Sydney 97 virus.  Finally, Cluster III contains the "Illinois 99" swine virus, with an HA closest to human viruses circulating in 1996. To date, most reported isolations of H3N2 from swine have been from the Illinois 99 and Texas 98 clusters (2)

The co-circulation of H3N2 and classical H1N1 viruses in swine herds in the United States since 1998 has lead to the formation of "second generation" reassortant H1 viruses (Table 1).  The first to be described was an H1N2 virus the result of a double reassortment between cH1N1 and the triple reassortant swine H3N2 viruses (8,9). This H1N2 reassortant contains HA, M, NP and NS derived from classical swine H1N1; and the avian PA and PB2 and the human PB1 and NA derived from H3N2 (Figure 1).  Another "second generation" reassortant virus emerged in 2000, a reassortant H1N1 (2). This virus is similar to the H1N2, except that the NA is from classical H1N1, rather than from the H3N2 virus (Figure 2). This reassortant H1N1 (rH1N1) virus cannot be distinguished from cH1N1 by the standard tests used diagnostically for subtype analysis. Genetic sequencing of the polymerase genes, to determine if they are swine or avian, is the only definitive way to distinguish between the cH1N1 and rH1N1 viruses.  The presence of the avian polymerases seems to have given these rH1N1 viruses a selective advantage, and they are now co-circulating along with cH1N1 within US swineherds (2). These newer rH1N1 and H1N2 viruses are also showing considerable antigenic variability, indicating that the presence of the avian polymerase genes may also be responsible for antigenic drift at a higher rate than was seen with the more stable classical H1N1 viruses (2,10,11). A recent survey of sera from the major swine-producing areas in the United States in 2000 indicates that 52.6% of sera had antibodies to H1N1 and 47.4% had antibodies to H3N2 (10). In this survey, a 1:40 titer was considered as the cutoff in the HI test so the actual prevalence may be higher.  Subtyping of 2001 isolates by reverse transcription PCR showed that 67.1% of isolates were H1N1, 10.8% were H1N2, and 38.3% were H3N2. Thus, these three SIV subtypes are co-circulating and widespread within US swineherds.

**Table 1.**  Origin of SIV genes in viruses circulating in North American swine

| SIV Virus | HA | NA | $M_1/M_2$ | NP | PA | PB1 | PB2 | $NS_1/NS_2$ |
|---|---|---|---|---|---|---|---|---|
| Classical H1N1 | swine | swine | swine | swine | swine | swine | swine | swine |
| "Texas 98" H3N2 | human 95 | human | swine | swine | avian | human | avian | swine |
| "Illinois 99" H3N2 | human 96 | human | swine | swine | avian | human | avian | swine |
| "Colorado 99"H3N2 | human 97 | human | swine | swine | avian | human | avian | swine |
| H1N2 | swine | human | swine | swine | avian | human | avian | swine |
| Reassortant H1N1 | swine | swine | swine | swine | avian | human | avian | swine |

**Figure 1:** Origin of genes of H1N2 viruses in the United States.
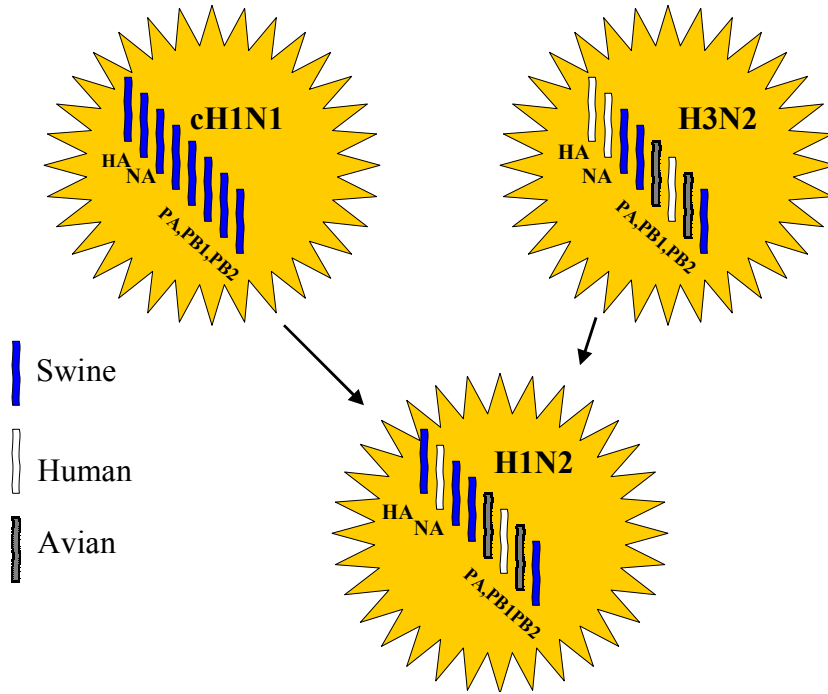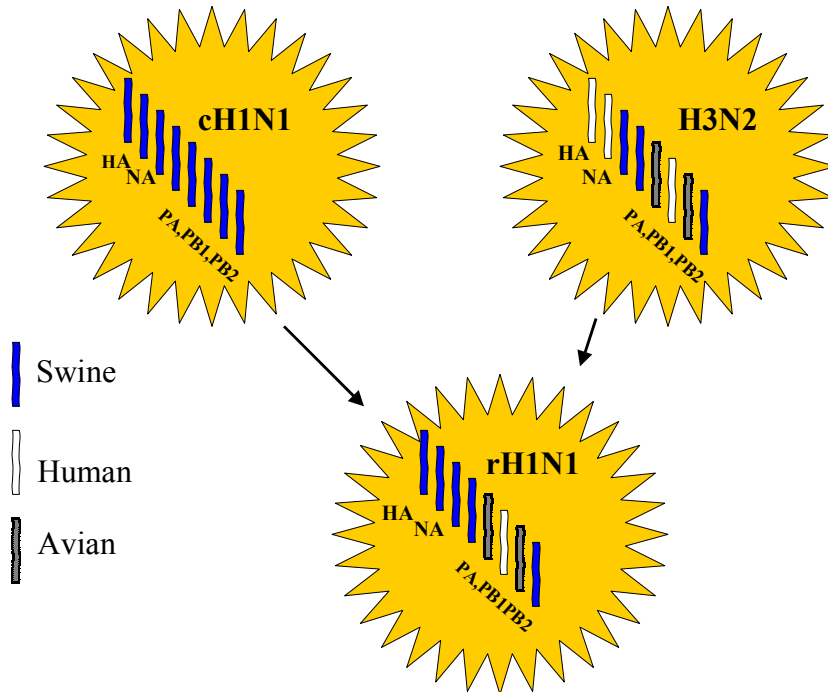
**Figure 2:** Origin of genes of reassortant H1N1 viruses in the United States.

## Epidemiology of European SIV

Although H1N1, H1N2, and H3N2 viruses are also the predominant subtypes circulating in Europe (Table 2), the origin of these viruses if very different from the origin of the North American viruses (12,13,14). Genetic reassortment and co-circulation of different subtypes in pigs occurred on the European continent much earlier than the appearance of the different lineages in North America. The H1N1 virus currently predominant in the European swine population is H1N1 virus that appeared in 1979, and this virus is a wholly avian virus (15). Unlike the situation in the US, where two H1N1 lineages are currently co-circulating, this "avian-like" H1N1 virus completed replaced the cH1N1 virus in Europe. An H3N2 virus also appeared in the early 1980's that was similar to the human pandemic "Hong Kong" viruses of 1968. Later studies showed that genetic reassortment between avian and human-like H3N2 viruses in European pigs had been occurring since 1983 (16). This reassortant virus, with human-like HA and NA genes and avian internal genes, is currently the predominant H3N2 virus circulating in swine in Europe. More recently, in Great Britain in 1994, an H1N2 reassortant was identified that probably arose from multiple reassortment events from 3 different viruses (13, 17). The HA of this H1N2 virus is derived from a human H1N1 virus and is genetically and antigenically very different from the avian H1N1 viruses. This H1N2 virus is also present on the European mainland (18). In 1999 it was shown that in Flanders, 100% of herds were seropositive to H1N1, 97% to H3N2, and 85% to H1N2 (4). Most pigs have antibodies to more than one subtype, indicating that all 3 viruses appear to be co-circulating and widespread in European swineherds.

Table 2. Origin of SIV genes in viruses circulating in European swine

| SIV Virus | HA | NA | M1/M2 | NP | PA | PB1 | PB2 | NS |
|---|---|---|---|---|---|---|---|---|
| H1N1 "avian like" | avian | avian | avian | avian | avian | avian | avian | avian |
| H3N2 "human like" | human 1968-1973 | human | avian | avian | avian | avian | avian | avian |
| H1N2 reassortant | human 1980-86 | human H3N2 | avian | avian | avian | avian | avian | avian |

## Clinical Implications of the New Swine Influenza Viruses

Vaccination is perhaps the most effective strategy to control SIV disease. Thus, the introduction on new SIV subtypes and lineages in both European and North American swine is a concern as far as the efficacy and cross-protection of vaccines containing older strains. The composition of the human influenza vaccine is evaluated each year and strains are updated based on epidemiological information available from a network of surveillance mechanisms. However, there is no similar mechanism in place for updating swine vaccines in Europe or in the United States, and changes to vaccine composition to assure cross-protection against contemporary SIV isolates cannot be made as quickly is done for humans. There is no doubt that antibodies to the HA glycoprotein are an important correlate to protective immunity, and HI titers of vaccinated swine against newer SIV isolates are a good method to evaluate antigenic differences that might indicate the need to change vaccine strains. However, protection has been demonstrated in the absence of high HI titers and other components of the innate, antibody, and cell-mediated immune response may also be important host defenses against SIV disease [reviewed in (21)].

In Europe, bivalent SIV vaccines containing A/Port Chalmers/1/73 (H3N2) and either A/New Jersey/8/76 (H1N1) or Sw/Netherlands/25/80 (H1N1) were first registered in the mid-1980's (19). In spite of differences between the H1N1 vaccines strain and the avian viruses present in European swine, this vaccine has been shown to be protective against challenge with newer H1N1 isolates from 1983 and 1998 (19, 20). It was concluded that the high antigen dose and potent adjuvant in the commercial vaccine elicited antibody titers that were high enough to overcome challenge with the heterologous viruses. Antigenic drift has also been detected in European H3N2 viruses and it has been recommended that the Port Chalmers vaccine strain be replaced with a more recent isolate (22). Evaluation of the efficacy of the commercial vaccine against challenge with a 1996 H3N2 field isolate demonstrated that the vaccine did protect against clinical disease and reduced virus shedding, compared to nonvaccinated controls (23). Cross-protection was attributed to a strong HI and virus neutralizing (VN) serum antibody response. However, the protection was not as strong as that elicited in pigs previously exposed to a live virus challenge. The pigs immunized

by pre-exposure to the live virus developed higher local IgA antibodies and a stronger cellular response as measured by lymphocyte proliferation, and these immune mechanisms may be important in cross-protection.

Since studies demonstrated that the current European vaccine did protect against more contemporary H1N1 and H3N2 viruses, a more important question is the ability of the vaccine to protect against the European H1N2 reassortant virus that was introduced in the mid 1990's. The HA and NA glycoproteins of the H1N2 virus are genetically and antigenically very different from those of the H1N1 and H3N2 viruses, and it was demonstrated that the commercial vaccine did not protect against experimental challenge with the H1N2 virus (24). Thus, inclusion of the H1N2 strain into the European vaccines should be considered. In contrast to protection after vaccination, pigs exposed to live H1N1 and H3N2 and subsequently challenged with H1N2 were fully protected (25). Interestingly, pigs pre-exposed only to H1N1 or H3N2 virus were not protected from H1N2 challenge. While the protected swine did not have high levels of HI or VN antibodies to H1N2, antibodies against the NA or other internal proteins may have been responsible for the cross-protection that was observed in this study. These studies showed that cross-protection within different lineages or even between subtypes (heterosubtypic protection) may be more evident in pigs that are exposed to live virus than those vaccinated with killed vaccines. It is possible that a combination of vaccination and natural field exposure may elicit a broader, more cross-protective response than that which can be demonstrated in experimental challenge models.

The first SIV vaccine for classical H1N1 was introduced in the United States in the early 1990's. After the emergence of the triple reassortant H3N2 viruses in 1998, several bivalent vaccines have been licensed (26). These vaccines are primarily used to vaccinate breeding stock, gilts prior to introduction into the breeding herd, and as a pre-farrowing vaccine. In spite of widespread vaccination, there are still reports of apparent vaccine breaks or failures in herds exhibiting acute outbreaks of respiratory disease involving SIV. In feeder pigs, this may be due in part to the presence of maternally-derived antibodies (MDA), that can block the pig's active immune response (21). However there has been a concern that some acute outbreaks in feeder pigs or breeding herds may be due to the emergence of new strains that are not protected by commercial vaccines, and some swine producers are using herd-specific or autogenous vaccines.

In the United States, the newer triple reassortant H3N2 viruses, represented by Clusters II and III, are antigenically and genetically distinct from the Texas-like Cluster I that first appeared (7,27,28). In addition, some differences in virulence between H3N2 isolates have been reported in experimental challenge studies (28, 29). Presently, Cluster I and III viruses are most prevalent in US swineherds. There have only been a couple of confirmed reports of isolation of Cluster II viruses, so this virus may not have the selective advantage of the other clusters. In a recent study, a vaccine prepared from the Texas-like lineage protected pigs from experimental challenge with an Illinois-like Cluster III virus that was isolated from an acute outbreak in a sow farm in North Carolina (27). The fact that there was only 92% identity of the HA gene of the vaccine and challenge viruses indicates that genetic sequence analysis is not always the best predictor of vaccine efficacy. It has also been demonstrated that a vaccine prepared with cH1N1 can protect against lung lesions and infection in pigs experimentally challenged with an H1N2 isolate from the US (30). This protection was evident even though the HI antibody response to H1N2 was very low in the vaccinates, indicating that mechanisms other than antibodies to the HA may be important in vaccine efficacy. The contemporary H1N1 isolates currently circulating in US swineherds exhibit considerable genetic and serologic diversity (11). Experimental studies to examine cross-protection between the cH1N1 and newer rH1N1 viruses have only recently been reported (31, 32). In these studies, vaccines formulated at a minimum protective dose based on efficacy against challenge with the homologous rH1N1 or cH1H1 lineage did not fully protect against challenge with a virus representing the heterologous lineage. There is no way to know whether the rH1N1 will eventually replace cH1N1, as happened after the introduction of the avian H1N1 in Europe, or whether these two lineages will continue to co-circulate. Thus, in the US, the broadest spectrum of protection against H1N1 would be with a multivalent vaccine containing both viruses.

In summary, vaccination against SIV will continue to be an important tool in the control of SIV disease. However, in both North America and Europe, the virus has shown be adept at changing its phenotypic characteristics in order to evade the host's immune defenses. Prediction of vaccine cross-protection against different lineages within a subtype is difficult, and cannot be based on genetic or serologic data alone. While experimental vaccination and challenge studies remain the gold standard for evaluating cross protection, extrapolation of the results to all field situations is also difficult, because pigs in the field are exposed to both vaccine and live infections, as well as the other bacterial and viral pathogens involved in PRDC. Maternal antibodies may be present for long periods in

piglets from vaccinated sows (33) and inactivated SIV vaccines do not effectively overcome inhibition by MDA. Thus, breaks in vaccine efficacy presumed to be due to the presence of heterologous strains may in fact be due to MDA, or due to immunosuppressive effects of other pathogens circulating on the farm. Currently, only killed, adjuvanted vaccines are licensed for use in swine in North America and Europe, and there are no mechanisms for evaluating and replacing vaccine strains regularly, as is done with the human vaccines. There is a need for more flexibility in the licensing processes, better SIV surveillance systems, and the development of newer vaccine technologies that can overcome MDA and produce a broader protective response, so that swine influenza infections can be more effectively controlled in the future.

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