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# Swine influenza vaccines: current status and future perspectives

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## Abstract

Swine influenza is an important contagious disease in pigs caused by influenza A viruses. Although only three subtypes of influenza A viruses, H1N1, H1N2 and H3N2, predominantly infect pigs worldwide, it is still a big challenge for vaccine manufacturers to produce efficacious vaccines for the prevention and control of swine influenza. Swine influenza viruses not only cause significant economic losses for the swine industry, but are also important zoonotic pathogens. Vaccination is still one of the most important and effective strategies to prevent and control influenza for both the animal and human population. In this review, we will discuss the current status of swine influenza worldwide as well as current and future options to control this economically important swine disease.

**Keywords:** swine influenza, vaccines

## Introduction

Swine influenza is one of the most important respiratory diseases in pigs. The recent pandemic H1N1 virus is genetically very similar to influenza viruses that occur in swine and has been transmitted from humans to other species including pigs. Control of virus spread among herds and prevention of possible transmission to humans can be achieved through the vaccination of swine. Even though only three subtypes of influenza A viruses, H1N1, H1N2 and H3N2 predominantly infect pigs worldwide, current commercially available inactivated vaccines for swine are not highly efficacious (at least in North America) due to the multitude of genetically diverse viruses co-circulating in swine herds today. Since swine are susceptible to both human and avian influenza viruses, viral reassortment can occur in pigs allowing the generation of novel viruses which might be the cause of a human pandemic (Brown, 2008). These are major concerns to both the swine industry and public health. Control of influenza infection in swine is critical not only for the reduction of disease symptoms and economic losses but also to limit potential viral reassortment, cross-species adaptation and the spread of influenza viruses. This review will discuss the current epidemiological situation of swine influenza virus (SIV) worldwide and the challenges faced by current commercially available inactivated vaccines. We will focus on various strategies to develop future swine influenza vaccines such as live-attenuated, subunit, vectored and DNA vaccines.

## Influenza A virus and swine influenza

Swine influenza is caused by influenza A virus, a genus within the family *Orthomyxoviridae*. Influenza A virus is a negative, single-stranded RNA virus whose genome consists of eight gene segments that encode 10 or 11 viral proteins. The glycoprotein hemagglutinin (HA) and neuraminidase (NA) are encoded by segments 4 and 6, respectively; both are located on the surface of the virus and are responsible for viral entry (HA) and efficient viral release from the infected cells (NA). In addition, these surface proteins are highly variable and are the major targets of the host humoral immune response. Segment 7 of influenza A viruses encodes the M1 and M2 proteins. The M2 protein is integrated into the viral envelope and its ion channel activity is required for efficient viral uncoating during virus invasion of cells. The M1 protein

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lies beneath the virus envelope and is thought to be critical for virus assembly and budding. The eight viral RNA segments together with the nucleoprotein (NP, segment 5) and the viral polymerase proteins, PB2 (segment 1), PB1 (segment 2) and PA (segment 3), form the ribonucleoprotein complex that participates in RNA replication and transcription. Segment 8 encodes for the non-structural protein 1 (NS1) and NS2 or nuclear export protein (NEP). The major function of the NS1 is to modulate the type I interferon (IFN) response of the host (Garcia-Sastre *et al.*, 1998). The viral NEP has been found in the virus particle and is required for the export of the viral RNA from the nucleus to the cytoplasm of infected cells (O'Neill *et al.*, 1998). In addition, PB1 using an alternative open reading frame in many influenza A viruses encodes a pro-apoptotic factor called PB1-F2 (Chanturiya *et al.*, 2004), which modulates virulence and severity of secondary bacterial infections (Zamarin *et al.*, 2006; Conenello *et al.*, 2007; McAuley *et al.*, 2007).

The most significant characteristic of influenza A virus is its enormous genetic variability, which presents an immense challenge in the control and prevention of disease. Two major mechanisms contribute to this: antigenic drift (random mutations within individual genes) and antigenic shift. Antigenic shift or reassortment occurs when two or more different influenza A viruses infect the same cell and a mixing of RNA segments results in novel reassortant viruses.

Influenza A viruses are divided into subtypes based on the antigenic nature of their HA and NA glycoproteins. Currently, 16 HA subtypes (H1–H16) and 9 NA subtypes (N1–N9) have been isolated from wild waterfowl and seabirds (Webster *et al.*, 2006; Wright *et al.*, 2007). Although aquatic birds are the major reservoir for influenza A viruses, pigs play an important role in the transmission of novel viruses to humans by acting as a 'mixing vessel' (Scholtissek, 1994; Brown, 2008; Ma *et al.*, 2009a), since human, avian and SIVs can replicate in pigs (Ito *et al.*, 1998; Ma *et al.*, 2009a).

## SIV worldwide

Swine influenza in pigs leads to fever, lethargy, sneezing, coughing, labored breathing and decreased appetite; it presents with high morbidity (approaching 100%) and generally low mortality (<1%) rates. Despite the low mortality in herds, it is still an economically important infectious disease for the swine industry. The following subtypes of influenza A virus predominantly infect pigs worldwide.

### *Classical H1N1 virus*

Swine influenza was first recognized in 1918 in the USA, Hungary and China, coinciding with the 1918 Spanish pandemic in humans (Webster, 2002). The first SIV isolate belonging to the H1N1 subtype was obtained in 1930 from U.S. pigs (Shope, 1931); subsequently, a similar virus was isolated from humans (Smith *et al.*, 1933). This H1N1 swine virus and closely related viruses are designated classical H1N1 (cH1N1) viruses. For the next 50 years, SIVs were almost exclusively cH1N1 virus in swine populations worldwide. The cH1N1 viruses began disappearing from the European pig populations after 1979 with the emergence of the avian-like H1N1 virus (Pensaert *et al.*, 1981). In North America, the cH1N1 virus was relatively conserved as the predominant virus until 1998 (Hinshaw *et al.*, 1978; Chambers *et al.*, 1991; Olsen *et al.*, 2000). To date, the cH1N1 viruses are still the predominant viruses in Asian pigs (Liu *et al.*, 2009).

### *Avian-like H1N1 virus*

In 1979, an avian-like H1N1 virus emerged in European swine populations. The virus is antigenically and genetically distinguishable from the cH1N1 SIVs (Scholtissek *et al.*, 1983; Brown *et al.*, 1997), and has quickly replaced the cH1N1 viruses in European pigs (Brown, 2000). All eight gene segments of this avian-like H1N1 prototype virus are directly derived from Eurasian avian influenza viruses without reassortment with other (human or swine) viruses (Dunham *et al.*, 2009). Currently, this avian-like virus is co-circulating in the European pig populations with swine influenza H3N2 and H1N2 subtypes. Recently, European avian-like H1N1 viruses have been isolated from pigs in China (Liu *et al.*, 2009; Yu *et al.*, 2009).

### *Reassortant H3N2, H1N2 and H1N1 viruses*

#### *Asia*

Following the 1968 H3N2 human pandemic, human-like H3N2 viruses and cH1N1 viruses were co-circulating widely in Asian and European pig populations (Kundin, 1970; Haesebrouck *et al.*, 1985), producing double reassortant H1N2 viruses through reassortment; the latter have become widespread in pigs and continue to circulate in pigs in Asia (Ouchi *et al.*, 1996; Jung and Chae, 2004; Qi and Lu, 2006). Recently, double reassortant H3N2 viruses containing human (HA and NA) and avian genes (PB2, PB1, PA, NP, M and NS) and triple reassortant H3N2 viruses carrying human (HA and NA), swine (NP) and avian (PB2, PB1, PA, M and NS) genes have emerged in pigs in China (Yu *et al.*, 2008). Novel triple reassortant H1N2 influenza viruses containing genes from the classical swine (HA, NP, M and NS), human (NA and PB1) and avian (PB2 and PA) lineages have been reported in pigs in China (Yu *et al.*, 2009).

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### *Europe*

With the replacement of cH1N1 viruses, the avian-like H1N1 has been the predominant virus in European pig populations and has undergone a reassortment with the human H3N2 virus, producing a human-like H3N2 virus containing HA and NA genes from the human virus and six internal genes from the avian-like virus (Castrucci *et al.*, 1993). Subsequently, an H1N2 virus, first isolated from Great Britain swine herds, spread to the rest of Europe (Van Reeth *et al.*, 2000). The H1N2 virus contained human-like H1 and N2 genes and avian-like internal genes (Brown *et al.*, 1998). In 2005, a novel H1N2, which was a reassortant between swine H1N2 and swine H3N2 virus, was identified in Germany (Zell *et al.*, 2008). Currently, the avian-like H1N1, human-like H3N2 and reassortant H1N2 SIVs have become widespread among pigs in Europe (Van Reeth *et al.*, 2008).

### *North America*

Since 1998, triple reassortant H3N2 viruses were isolated from pigs and have been endemic in swine herds of North America; they contain HA, NA and PB1 polymerase genes from human influenza viruses, M, NS and NP genes from classical swine viruses, and PA and PB2 polymerase genes from avian viruses (Zhou *et al.*, 1999; Webby *et al.*, 2000). Reassortment between triple reassortant H3N2 viruses and cH1N1 viruses has resulted in the subsequent development of H1N2 (Karasin *et al.*, 2000), reassortant H1N1 (rH1N1) (Webby *et al.*, 2004) and H3N1 viruses (Lekcharoensuk *et al.*, 2006; Ma *et al.*, 2006). The rH1N1 viruses contain the HA and NA from the cH1N1 virus and the internal genes from triple reassortant H3N2 viruses. The H1N2 viruses contain the HA from the classical swine virus and the NA and internal genes from the triple reassortant H3N2 viruses (Karasin *et al.*, 2002; Webby *et al.*, 2004). The H3N1 viruses contain the NA from the classical swine virus and the HA and internal genes from the triple reassortant H3N2 viruses. Also, novel human-like H1N1 and H1N2 SIVs have been isolated from swine herds across the U.S., representing a reassortment of triple reassortant SIVs with seasonal human H1N1 viruses; therefore, the HA and/or NA genes are human-like whereas the internal genes are derived from triple reassortant SIVs (Vincent *et al.*, 2009). The reassortant H3N2, H1N2 and H1N1 (including rH1N1 and human-like H1N1) viruses are circulating in swine populations in North America (Vincent *et al.*, 2008b; Ma *et al.*, 2009b).

## Immunity to influenza A viruses

Infection of influenza A virus triggers immune responses of the host including innate immunity, mucosal immunity and systemic immunity (both humoral and cell-mediated immunity). Innate immunity is the first line of host defense inhibiting influenza virus replication in a non-specific manner and is therefore critical in the early containment of influenza virus infection (White *et al.*, 2008). The innate immune response is complex involving a variety of soluble innate inhibitors in respiratory secretions and strongly contributes to the promotion and direction of the adaptive, pathogen-specific immune response (White *et al.*, 2008; McGill *et al.*, 2009). There are several excellent reviews on influenza innate immunity (Ichinohe *et al.*, 2008; White *et al.*, 2008; McGill *et al.*, 2009). To start an infection, influenza viruses first attach to the mucosal tissues of the respiratory tract. Cellular recognition of viral products such as viral RNA by Toll-like receptors or cytoplasmic sensors (e.g. retinoic acid-inducible gene I and melanoma differentiation-associated gene 5) results in induction of the type I IFN system to establish an antiviral state in the cell.

If animals were previously exposed or vaccinated against influenza viruses, the mucosal immune response provides an important line of defense against influenza infection apart from innate immunity. Specific IgA and IgM secreted locally in the respiratory tract are the major neutralizing antibodies that prevent influenza virus entry and can inhibit influenza replication intracellularly (Cox *et al.*, 2004). The neutralizing antibodies detected in nasal secretions specifically target the HA and NA surface proteins of influenza virus. In the pig model, influenza-specific mucosal antibodies have been detected and demonstrated to contribute significantly to the clearance of SIV from the respiratory tract (Larsen *et al.*, 2000; Richt *et al.*, 2006). Mucosal immunity induced by natural influenza infection at the respiratory tract is more effective and protective against subsequent heterovariant virus infection than systemic immunity induced by parenteral immunization with inactivated vaccines (Ichinohe *et al.*, 2008).

During infection, the humoral immune system produces antibodies against all major influenza viral proteins. Antibody to the HA is the most important for neutralization of virus and therefore prevention of disease. In contrast, antibody to the NA is less effective in preventing infection, but it prevents the release of mature viruses from infected cells. Antibodies to the conserved internal proteins (M and NP) cannot provide protection from infection (Cox *et al.*, 2004; Wesley *et al.*, 2004), although there could be a role for the M2 protein in antibody-mediated protection (Treanor *et al.*, 1990; Wang *et al.*, 2008). The HA- and NA-specific antibodies in serum are most important for protection against influenza; therefore, the serum antibody level to HA and NA are considered to correlate with the prevention and resistance to illness (Cox and Subbarao, 1999). However, the humoral immune response might fail to prevent influenza infections if faced with antigenic shift and/or drift of the infecting virus.

Cell-mediated immunity is believed to play an important role in clearance of influenza viruses from the

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respiratory tract and subsequent recovery from disease. Influenza-specific cytotoxic T lymphocytes (CTLs) have been found in the blood and the lower respiratory tract of infected hosts and are able to lyse cells infected with different subtypes of influenza A virus. In mice and humans, specific CTL response is directed against influenza viral internal proteins, specifically against NP, M1, NS1 and the polymerase proteins (PB1, PB2 and PA) (Bennink *et al*., 1982, 1987; Gotch *et al*., 1987; Reay *et al*., 1989; Jameson *et al*., 1998; Epstein *et al*., 2000). The NP of influenza A viruses is an important target antigen for both subtype-specific and cross-reactive CTLs in mice and humans (Townsend *et al*., 1984; Yewdell *et al*., 1985; McMichael *et al*., 1986). There is limited knowledge on cellular immune responses in pigs after influenza infections (Heinen *et al*., 2001). Previous studies indicate that the CTL response is cross-reactive between influenza A strains providing heterovariant and heterosubtypic immunity and is critical in reducing viral spread and clearing virus in combination with neutralizing antibodies (Nguyen *et al*., 2001). Therefore, an ideal vaccine is able to induce a balanced immune response including mucosal, humoral and cell-mediated immunity.

## Swine influenza vaccines

Although pigs are susceptible to infection with many subtypes of influenza A viruses (Kida *et al*., 1994), only three subtypes (H1N1, H1N2 and H3N2) are consistently isolated from swine herds worldwide (Webster *et al*., 1992; Olsen, 2002; Landolt and Olsen, 2007). Despite this limited repertoire of circulating subtypes, novel genotypes within individual subtypes and novel reassortant viruses (e.g. human-like H1N1) have been an enormous challenge for the production of efficacious vaccines to prevent and control swine influenza. Antigenic shift and drift of SIVs are occurring constantly, and the present system for the production and licensing of inactivated SIV vaccines does not allow the industry to react in a timely manner. To date, only inactivated whole-virus vaccines are commercially available and widely used for swine influenza worldwide.

### *Inactivated SIV vaccine*

Current commercially available SIV vaccines are traditional, adjuvanted, inactivated bivalent whole-virus vaccines containing H3N2 and H1N1 subtype SIVs propagated in embryonated hen eggs. These vaccines stimulate high titers of IgG in serum and lungs, which are critical for ameliorating or preventing influenza virus infection and protection against clinical disease. However, protection is to be expected only when the priming HA antigen is antigenically matched or closely related to the HA of the challenge virus. Since there is great genetic and antigenic variety within currently circulating SIVs, commercially available vaccines are not able to provide optimal protection for pigs against SIVs. A number of studies have shown only partial protection from inactivated virus vaccines following a heterovariant or heterosubtypic influenza challenge (Brown and McMillen, 1994; Bikour *et al*., 1996; Vincent *et al*., 2010a); they are only efficacious when genetically similar viruses are used for challenge. Other studies have revealed that previous exposure of pigs to European H1N1 and H3N2 viruses conferred complete protection against a novel H1N2 with an unrelated HA protein (Van Reeth *et al*., 2003). In contrast, vaccination with commercially available inactivated vaccines containing H1N1 and H3N2 viruses does not protect against the H1N2 challenge (Van Reeth *et al*., 2004), indicating that serum hemagglutination inhibition (HI) or virus neutralizing antibodies are not essential and that cell-mediated and/or mucosal immunity are critical for heterosubtypic protection. One study has shown that a killed cH1N1 SIV vaccine not only fails to protect against a heterologous H1N2 infection but surprisingly also potentiates pneumonia in challenged pigs (Vincent *et al*., 2008a). These results indicate that inactivated vaccines when faced with a heterovariant challenge may enhance disease.

Interference by maternally derived antibodies (MDAs) is another big challenge for vaccine (especially inactivated vaccines) efficacy because passively acquired antibodies from the sow's colostrum can inhibit the immunogenicity of a vaccine and interfere with the pig's immune response to the vaccine if they are still present at the time of immunization. Kitikoon *et al*. (2006) have shown that the MDA suppressed serum antibody responses and the induction of SIV-specific memory T-cells following the administration of a bivalent inactivated vaccine in pigs. Enhancement of lung pneumonia was observed in pigs immunized with a bivalent inactivated SIV vaccine in the presence of MDA when challenged with a heterologous H1N1 virus (Kitikoon *et al*., 2006).

In summary, there are three major difficulties with the use of current commercially available inactivated SIV vaccines: (1) SIV is antigenically changing faster than traditional inactivated vaccines can be developed; (2) the commercially available inactivated SIV vaccines do not provide good cross-protection among different SIV isolates, especially against heterovariant and heterosubtypic viruses; and (3) passively acquired immunity (MDA) can interfere with vaccine immunity in piglets. These difficulties have led to a significant decrease in the sale of commercially available SIV vaccines and a significant increase in the production and use of autogenous vaccines (presently about 50% of the U.S. market). A priority for novel SIV vaccine development is the improvement of heterovariant and heterosubtypic immunity and the selection of currently circulating SIV isolates as vaccine seeds.

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## Live-attenuated swine influenza as vaccines

With the development of molecular biology technology, influenza viruses can be rescued from plasmid DNA by a technique called reverse genetics. This method makes it possible to modify the viral genome for generation of rationally designed novel live-attenuated influenza virus vaccines as described in the following section. The surface glycoprotein HA of influenza A virus mediates virus entry into susceptible cells. HA is synthesized as a precursor HA0 comprising HA1 and HA2. Cleavage of the HA0 into HA1 and HA2 by host proteases is a prerequisite to gain access to cells by activating the fusion peptide; this process is a major determinant of virus pathogenicity. The cleavage site contains a conserved arginine or a multiple basic amino acid motif. Mutation of the HA cleavage site, which now requires cleavage by elastase instead of trypsin, has led to the attenuation of influenza viruses in mice (Stech *et al.*, 2005). The polymerase proteins PB2, PB1 and NP have been shown to contribute to the virus ability to grow at a lower temperature in some temperature-sensitive virus strains (Jin *et al.*, 2003). The NS1 protein of the influenza A virus is exclusively expressed in virus-infected cells and not present in virus particles. One of the major functions of the NS1 protein of influenza viruses is the inhibition of the innate host type I IFN-mediated antiviral response. Modifications of either the HA, the polymerase proteins PB1 and PB2, or the NS1 can be utilized to produce live-attenuated SIVs which have a great potential as live-attenuated vaccines. The advantage of modified live-attenuated vaccines is enhanced stimulation of cell-mediated immunity, directed most likely against the conserved NP (Yewdell *et al.*, 1985), thus providing more heterovariant and heterosubtypic protection (Xie *et al.*, 2009). A major concern with live-attenuated vaccines would be a possible reassortment between field viruses and vaccine strains, producing novel reassortant viruses.

### *Live-attenuated swine influenza vaccine with modified NS1 protein*

Attenuated SIVs expressing NS1-truncated proteins with 73, 99 or 126 amino acids (Tx/98 NS1△73, Tx/98 NS1△99 and Tx/98 NS1△126) with promising vaccine potential have been generated via modification of the viral NS1 gene of an H3N2 (A/Swine/Texas/4199-2/98, Tx/98) virus using reverse genetics (Solorzano *et al.*, 2005). The Tx/98 NS1△126 virus is the most attenuated virus displaying the lowest level of NS1 expression and decreased replication *in vitro* and *in vivo* compared to the wild-type and Tx/98 NS1△73, Tx/98 NS1△99 viruses (Solorzano *et al.*, 2005). Intratracheal infection of pigs with Tx/98 NS1△126 virus induces minimal macroscopic and histopathologic lung lesions. Pigs vaccinated with Tx/98 NS1△126 virus were completely protected against a challenge with the homologous Tx/98 virus and partially protected against a challenge with a heterosubtypic H1N1 virus (Richt *et al.*, 2006). All vaccinated pigs developed a detectable level of HI titers, serum IgG, and mucosal IgG and IgA antibodies against parental H3N2 antigens (Richt *et al.*, 2006).

Subsequent studies showed that the intranasal route was more efficient than the intramuscular route at eliciting mucosal anti-influenza virus antibodies (Vincent *et al.*, 2007). A single dose of Tx/98 NS1△126 virus administered intranasally conferred complete protection against a homologous virus challenge and nearly complete protection against a heterovariant challenge with an antigenically distant H3N2 SIV (A/Sw/CO/23619/99). Moreover, intranasal vaccination reduced clinical symptoms (fever) and virus titers in lungs of pigs which were challenged with a heterosubtypic H1N1 SIV (A/Swine/Iowa/00239/2004). These studies indicate that a complex host response including both cellular and humoral mechanisms contributes to the broad efficacy of the Tx/98 NS1△126 modified live virus (MLV) after intranasal delivery, and this efficacy appears to be superior to that induced by inactivated influenza vaccines (Vincent *et al.*, 2007). In addition, a study using the Tx/98 NS1Δ126 virus as an MLV vaccine in piglets with MDA revealed that Tx/98 NS1Δ126 virus can provide good immunity against homologous and heterovariant viruses without disease enhancement (Vincent *et al.*, 2010b). The series of experiments described above demonstrate that the NS1-truncated MLV vaccine appears to be more efficacious when compared to the inactivated vaccine, indicating that they are promising vaccine candidates against SIVs.

### *Elastase-dependent live attenuated swine influenza vaccine*

Avian- or mouse-adapted influenza viruses can be attenuated by modification of the HA cleavage site from a trypsin-sensitive motif to an elastase-sensitive motif. Recently, Masic *et al.* (2009a) used the same strategy to generate two elastase-dependent mutant SIVs derived from A/Sw/Saskatchewan/18789/02 (H1N1) called A/Sw/Sk-R345V (R345V) and A/Sw/Sk-R345A (R345A). These two viruses displayed similar growth properties *in vitro* to the wild-type virus, but were highly attenuated in pigs. This was demonstrated by significantly decreased macroscopic lung lesions and virus titers in lungs and no nasal virus shedding (Masic *et al.*, 2009a) when compared to the wild-type virus. Administration of either the R345V or R345A via an intratracheal route induced antigen-specific humoral and cell-mediated immunity. Pigs immunized with the R345V virus had significantly higher HI titers than the R345A-vaccinated animals (Masic *et al.*, 2009b). Therefore, the R345V virus was selected to further test its efficacy against a challenge from homologous and heterologous viruses. After pigs were vaccinated and boosted with this virus intratracheally, they were subsequently challenged with either the wild-type homologous A/Sw/Saskatchewan/18789/02 (H1N1), heterovariant A/Sw/Indiana/1726/88 (H1N1) or heterosubtypic Tx/98

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H3N2 virus. Pigs vaccinated with R345V virus were completely protected against a challenge with the homologous and heterovariant H1N1 SIVs and partially protected against a challenge with the heterosubtypic H3N2 SIV. This protection was measured by significantly reduced macroscopic and microscopic lung lesions, lower virus titers from the respiratory tract, and lower levels of pro-inflammatory cytokines (Masic *et al.*, 2009b). It can be concluded that elastase-dependent SIV mutants are promising candidates as live-attenuated virus vaccines against SIVs in pigs; however, the safety (reversion and re-assortment) and efficacy employing practical vaccination routes need to be further investigated.

### *Cold-adapted live attenuated vaccine*

Cold-adapted (temperature sensitive, *ts*) influenza viruses replicate efficiently at a cooler temperature (25°C or 26°C), but their growth is restricted at normal body temperature. The nature of *ts* live-attenuated viruses leads to their efficient multiplication in the cooler environment of the upper respiratory tract where they induce local and systemic immune responses, and inefficient replication in the warmer environment of the lower respiratory tract where wild-type viruses may cause severe lung damage. A *ts* influenza vaccine (FluMist®) has been approved in the U.S. for intranasal use in humans (Belshe, 2004) and a *ts* modified-live equine influenza virus vaccine (Flu Avert® I.N. Vaccine) derived from the wild-type A/Eq/Kentucky/1/91 (H3N8) influenza virus has been licensed and is commercially available in North America (Paillot *et al.*, 2006).

FluMist® vaccine strains contain six internal gene segments (PB1, PB2, PA, NP, M and NS) from the master donor virus (MDV), a cold-adapted human H2N2 (A/Ann Arbor/6/60) influenza virus, along with two external surface gene segments (HA and NA) derived from currently circulating human influenza viruses. The cold-adapted MDV for influenza A strains of FluMist® was generated using serial passages in primary chicken kidney cell culture at a temperature gradually reduced to 25°C (Maassab, 1967). Molecular analysis showed that the PB1, PB2 and NP protein of the MDV each contributes to viral temperature sensitivity and the combination of all three gene segments results in the expression of the *ts* phenotype of the MDV (Jin *et al.*, 2003). Site mutagenesis analysis revealed that five loci [PB1 (K391E, E581G, A661T), PB2 (N265S) and NP (D34G)] are responsible for the *ts* phenotype of the MDV (Jin *et al.*, 2003).

Flu Avert® vaccine derived from A/Eq/Kentucky/1/91 (H3N8) influenza virus was created by serial passage in embryonated chicken eggs at consecutively lower temperatures (Youngner *et al.*, 2001). This *ts* vaccine replicates efficiently at 26°C while its growth is restricted at 38°C and 39°C. Intranasal immunization of ponies with a single dose of Flu Avert® provided full protection from clinical signs of the disease following a challenge with the parental A/Eq/Kentucky/1/91 influenza virus at 5 weeks and 6 months post vaccination. At late time points post-immunization, the duration of nasal virus shedding was significantly shorter when compared to unvaccinated control ponies (Townsend *et al.*, 2001). This vaccine also protects horses against infection with a heterovariant equine influenza virus (Chambers *et al.*, 2001). *Ts* live-attenuated equine influenza vaccines elicit long-term immunity that ameliorates duration and severity of clinical signs and nasal shedding of virus after challenge.

Solórzano *et al.* (2010) have generated an attenuated *ts* H3N2 SIV (A/Swine/WI/14094/99; Sw99) by changing the viral PB1 and PB2 genes (four loci) based on sequences observed in the cold-adapted human H2N2 (A/Ann Arbor/6/60) influenza virus. The mutated virus, called Sw99ts, was partially attenuated *in vitro* and *in vivo*. In order to make a fully attenuated virus, the PB1 and PB2 mutations were combined with the insertion of an HA epitope (eight amino acids derived from the influenza virus H3 HA protein sequence) into the C-terminus of the PB1 protein. The virus, named Sw99att, showed no replication at the restrictive temperature (39°C) but replicated efficiently at 33°C in cell culture. To show the potential of Sw99att as a live-attenuated vaccine virus, the surface genes were substituted with the HA and NA genes of a cH1N1 (A/Swine/IA/15/30, IA30) virus. Vaccination of mice with this virus provided complete protection in a homologous challenge (IA30) and partial protection (no clinical signs) following a heterovariant challenge with the 2009 pandemic H1N1 virus. The potential of this *ts* live-attenuated vaccine for pigs needs to be evaluated (Solórzano *et al.*, 2010).

## *Baculovirus-derived influenza subunit vaccines*

The baculovirus-insect cell expression system was developed over 20 years ago and has become one of the most widely used systems for production of recombinant proteins for both veterinary and human vaccines. The baculovirus system is a good method for producing recombinant glycoprotein due to the eukaryotic nature of the insect cells. Currently, more than 10 baculovirus expression system-derived vaccines are either commercially available (e.g. classical swine fever, porcine circovirus associated disease) or in clinical trials (hepatitis B and C viruses) (Meghrous *et al.*, 2009). In addition to the ease and safety of production, this system is an ideal platform for producing recombinant proteins owing to effective post-translational modification and high yields (He *et al.*, 2009; Meghrous *et al.*, 2009). Therefore, recombinant influenza proteins produced by the baculovirus expression system used as a subunit vaccine might be an alternative strategy to overcome the limitations and drawbacks of traditional killed influenza vaccines produced by the egg-based manufacturing system. The main advantage of a subunit vaccine derived from the

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baculovirus expression system is that the manufacturing of the HA proteins does not require the handling of live influenza viruses as required for embryonated eggs or mammalian cell production systems (Cox and Hollister, 2009).

Numerous studies have been conducted on the immunogenicity and safety of baculovirus expression system-derived recombinant HA vaccines in the last two decades. A recombinant HA influenza vaccine provided equivalent or better immunogenicity than an egg-derived inactivated vaccine and was safe and efficacious in human clinical trials (Treanor *et al*., 1996, 2006, 2007; King *et al*., 2009). Subunit HA vaccines for avian and human influenza viruses have been studied in animal models (Powers *et al*., 1997; Crawford *et al*., 1999; Gambotto *et al*., 2008) and some are in clinical trials (Powers *et al*., 1997; He *et al*., 2009). However, to our knowledge, no baculovirus-derived subunit vaccines based on SIV antigens have been produced and tested in the pig model. One disadvantage of this technology is that it produces a highly hydrophobic recombinant HA protein, which makes purification difficult, resulting in a decrease of its effectiveness as a vaccine (He *et al*., 2009). In addition, a large amount of recombinant HA is required for vaccination in order to achieve an equivalent immune response to that of traditional inactivated influenza vaccines (Gambotto *et al*., 2008). The biggest challenge for subunit vaccines derived from the baculovirus system is the frequent antigenic drift and/or shift of the HA, leading to a mismatch between the immunogen and circulating viruses (Carrat and Flahault, 2007) and therefore vaccine failure.

## Vectored vaccines

A vector is a biological carrier of genes of other pathogens. The viral antigens expressed by vectored vaccines are produced in host cells *in vivo* and can induce both humoral and cellular immunity. The following vectored vaccines for swine influenza are currently under investigation or might be studied in the near future.

### *Adenovirus-based SIV vaccine*

A human adenovirus serotype 5 (hAd5) vector has been utilized to express various genes of interest for molecular therapy and vaccine development. Vaccination with human adenovirus vectors induced both humoral and cell-mediated immunity, making them potentially more effective than inactivated or subunit vaccines and more similar to the response elicited from MLV vaccines (Gamvrellis *et al*., 2004). In addition, administration of hAd5 vectored vaccines via the mucosal route induced superior, long-lasting mucosal immunity (Baca-Estrada *et al*., 1995). hAd5 viruses have broad host ranges and accommodate large segments of foreign DNA. Normally, livestock does not have pre-existing immunity against hAd5 virus that can interfere with vaccine efficacy (Wesley *et al*., 2004). A series of studies have shown that certain disadvantages of inactivated vaccines can be overcome by using recombinant hAd5-vector vaccines that can stimulate cell-mediated and mucosal immunity (Baca-Estrada *et al*., 1995; Monteil *et al*., 2000; Wesley *et al*., 2004).

A hAd5 recombinant virus expressing the HA of the H3N2 Tx/98 virus has shown partial protection in mice after a challenge with a heterovariant virus, A/HK/1/68 (H3N2) (Tang *et al*., 2002). Subsequently, a hAd5 recombinant virus expressing the NP of the Tx/98 H3N2 SIV was also generated and challenging experiments in pigs were conducted to test the efficacy of both hAd5 recombinant viruses as SIV vaccines (Wesley *et al*., 2004). Pigs vaccinated with the recombinant hAd5 expressing HA alone or HA plus NP developed high levels of virus-specific HI antibody by 4 weeks post vaccination, whereas the administration of the recombinant hAd5 expressing NP alone induced no detectable HI antibody. Pigs immunized with both recombinant viruses (HA and NP) in a mixture were completely protected as demonstrated by a lack of nasal virus shedding and lung lesions following a homologous challenge. Vaccination with the recombinant virus expressing HA induced nearly complete protection with a low viral titer in nasal swabs and minimal lung lesions. In contrast, vaccination with the recombinant virus expressing NP only reduced lung lesions when compared to non-vaccinated controls.

Subsequent studies demonstrated that the recombinant hAd5-vectored SIV vaccines are able to prime the immune system in the presence of MDA that often interfere with conventional inactivated vaccines (Wesley and Lager, 2006). Piglets with H3N2-specific MDA were either sham-immunized with an empty hAd5 vector or immunized with recombinant hAd5 SIV vaccines expressing the HA and NP. The HI titer of sham-immunized animals displayed continued antibody decay whereas piglets vaccinated with the recombinant hAd5 SIV vaccine developed an active immune response by the second week post vaccination. When the HI titer of sham-immunized piglets had decayed, the sham-immunized group and half of hAd5 SIV vaccinates were boosted with a commercial inactivated SIV vaccine and subsequently challenged with a heterovariant virulent H3N2 SIV. The pigs primed with the hAd5 SIV vaccine in the presence of MDA had a strong anamnestic response to the booster immunization while sham-immunized pigs did not respond to the commercial inactivated vaccine. The pigs primed with the hAd5-SIV vaccine and boosted with inactivated vaccine had a reduction of clinical signs, reduced virus loads in the respiratory tract and no lung lesions. In contrast, MDA positive pigs immunized with the inactivated vaccine alone exhibited a vaccine failure (Wesley and Lager, 2006).

In addition, the route of administration (needle-free device versus traditional intramuscular injection) for the

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recombinant hAd5 SIV vaccines was evaluated (Wesley and Lager, 2005). The results showed that a traditional intramuscular injection induced consistently higher HI responses than vaccination via a needle-free device, but the differences were not significant. Administration of high doses of the recombinant hAd5 SIV vaccine (HA and NP) using either method prevented nasal shedding after challenge. In these experiments, the hAd5 SIV vaccine virus was not transmitted to sentinel pigs (Wesley and Lager, 2005).

### *Alphavirus-based SIV vaccines*

Alphaviruses are positive-stranded RNA viruses belonging to the family of *Togaviridae*. Although alphaviruses have a broad host range including humans, their seroprevalence in many mammalian host species is rather low, making them potentially useful for vaccine development (Rayner *et al*., 2002). Three alphavirus [Sindbis virus (SINV), Semliki Forest virus (SFV) and Venezuelan equine encephalitis virus (VEEV)] replicon expression vectors have been developed (Xiong *et al*., 1989; Liljestrom and Garoff, 1991; Pushko *et al*., 1997). VEEV has a unique lymph node tropism (Walker *et al*., 1976; Jackson *et al*., 1991), resulting in effective antigen presentation and induction of a strong and balanced immune response. Alphavirus replicon expression vectors are propagation-defective (without alphavirus structural genes), single-cycle vectors incapable of spreading from infected to non-infected cells. However, these replicons are self-replicating and can efficiently express foreign antigens (Rayner *et al*., 2002). To date, numerous vaccine candidates based on alphavirus replicons have been developed and shown to induce protection against a variety of infectious pathogens in a number of hosts (Rayner *et al*., 2002).

Alphavirus replicon vectors have also been utilized to express influenza antigens and their efficacy in animal models was evaluated. Immunization of mice with SFV based-replicons expressing the NP and HA of influenza A virus provided protection against a challenge with the homologous virus (Berglund *et al*., 1999). A VEEV replicon vector has been used to express HA from the human Hong Kong H5N1 influenza A isolate (A/HK/156/97) and shown to protect chickens against a challenge with the homologous H5N1 virus (Schultz-Cherry *et al*., 2000). So far, Sagiyama virus is the only alphavirus found in swine and is geographically restricted to Asia (Chang *et al*., 2006). Importantly, pigs can be infected by VEEV and display a transient viremia (Dickerman *et al*., 1973), suggesting that the VEEV replicon vector can be used to develop vaccine candidates for the swine industry. Recently, a VEEV replicon vector expressing the HA from a human influenza virus A/Wyoming/03/2003 (H3N2) was developed to immunize pigs (Erdman *et al*., 2010). The results revealed that this VEEV replicon vector induced a robust HI antibody response in vaccinated pigs. The VEEV replicon vector has also been used to express the HA of the pandemic H1N1 A/California/04/2009 virus. Pigs were vaccinated and challenged with the homologous pandemic virus. Vaccinated pigs showed a significantly higher specific antibody response, reduced lung lesions and viral shedding, and higher average daily weight gain compared to non-vaccinated control infected animals, indicating that the VEEV replicon vaccine is efficacious for swine against the pandemic H1N1 virus (Vander Veen *et al*., 2009). This VEEV replicon vaccine has obtained a conditional license in the USA. Although the VEEV replicon particles can induce humoral, cell-mediated and mucosal immune responses and provide protection for a number of infectious agents, it is not clear whether this vector expressing an HA molecule can protect against heterovariant and heterosubtypic influenza viruses.

### *Pseudorabies virus (PRV)-based SIV vaccines*

PRV is an alpha-herpesvirus with a linear double-stranded DNA. PRV has a broad host range and its large DNA genome is capable of accommodating a large segment of foreign DNA. The PRV genome consists of many non-essential regions, such as genes encoding thymidine kinase (TK), gE, gG and gC, which can be deleted or replaced by other genes without affecting virus replication. For example, a commercially available attenuated DIVA (differentiating infected from vaccinated animals) vaccine for PRV containing a gE deletion has been widely used in the PRV eradication program (van Oirschot *et al*., 1986; White *et al*., 1996; Muller *et al*., 2003). Due to its good safety record and broad host spectrum, PRV is a promising vaccine vector for expressing antigens of choice from other pathogens (Thomsen *et al*., 1987; Whealy *et al*., 1988; van Zijl *et al*., 1991; Tian *et al*., 2006; Yuan *et al*., 2008) including the HA from SIVs. To express the HA of an H3N2 SIV (A/Swine/Inner Mogolian/547/2001), the PRV Bartha-K61 vaccine strain was utilized (Tian *et al*., 2006). Mice were immunized with this recombinant PRV expressing HA and challenged with a heterovariant H3N2 SIV at 4 weeks post vaccination. Vaccinated mice showed HI antibodies, reduced lung lesions, and an absence of virus from the lungs when compared to non-vaccinated control infected animals.

These results demonstrate that the recombinant PRV expressing SIV HA gene can protect mice from a heterovariant challenge and might be used as a candidate vaccine against SIV (Tian *et al*., 2006). However, the efficacy of a recombinant PRV as a swine influenza vaccine needs to be further evaluated in pigs. The major disadvantage of recombinant PRV as an SIV vaccine is that it might interfere with the surveillance for the control and eradication of PRV, because PRV has been eradicated from many countries.

### *Vaccinia virus-based vaccines*

Vaccinia virus is a large, complex, enveloped double-stranded DNA virus belonging to the poxvirus family.

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Vaccinia virus is well known as the vaccine used to eradicate human smallpox. The modified vaccinia Ankara (MVA) is a highly attenuated vaccinia strain created by serial passages in chicken embryo fibroblast cells and has been safely and successfully used to vaccinate over 120,000 humans against smallpox. The MVA has been engineered as a viral vector expressing foreign genes under control of a vaccinia virus promoter. To date, various recombinant MVA viruses have been shown to be immunogenic and induce protective immunity against viruses, bacteria and parasites in animal models and clinical trials (Sutter *et al.*, 1994; Moss *et al.*, 1996; Goonetilleke *et al.*, 2003; Moorthy *et al.*, 2003; Cosma *et al.*, 2003, 2007; Bisht *et al.*, 2004; Drexler *et al.*, 2004; McShane *et al.*, 2004; Wang *et al.*, 2004). The MVA has an excellent safety profile, the ability to elicit highly effective virus neutralizing antibody responses, is highly stable and replication deficient. It can be produced on a large scale in chicken embryo fibroblasts under BSL-1 conditions. Pre-existing immunity is unlikely to affect the immunogenicity of foreign antigens delivered by this vector (Ramirez *et al.*, 2000; Drexler *et al.*, 2004).

Recombinant MVA has been used to develop influenza vaccines by expressing various antigens from influenza A viruses. The MVA expressing the HA from H3N8 (A/Equine/Kentucky/1/81) equine influenza virus induced protective immunity in horses whereas vaccination with MVA expressing the NP provided very limited protection from clinical disease (Breathnach *et al.*, 2006). Vaccination of mice (two times $10^8$ pfu/dose) with MVA expressing the HA from the pandemic H5N1 (A/Vietnam/1194/04) virus induced protective immunity against infection with homologous and antigenically distinct heterovariant H5N1 (A/Indonesia/5/05; A/Hongkong/156/97) viruses (Kreijtz *et al.*, 2007). Subsequent studies showed that this recombinant MVA provided cross-clade protection in mice after a single immunization when challenged with different H5N1 viruses at low doses ($10^5$ pfu/dose) (Kreijtz *et al.*, 2009a). In addition, the H5 MVA vaccine induced cross-reactive antibodies and prevented virus replication in the upper and lower respiratory tract and the development of severe necrotizing bronchointerstitial pneumonia in H5N1 infected macaques (Kreijtz *et al.*, 2009b, c). Since MVA can be used to express the HA from influenza A viruses, it might be a promising future influenza vaccine (Rimmelzwaan and Sutter, 2009). However, to our knowledge no studies have been reported on a MVA-based SIV vaccine.

### *Virus-like particle (VLP) vaccines*

VLPs as vaccines have been discussed as promising alternatives for a variety of animal viral pathogens (Antonis *et al.*, 2006; Elmowalid *et al.*, 2007; Yang *et al.*, 2008) and are approved as vaccines in humans (Keating and Noble, 2003; Reisinger *et al.*, 2007). Influenza VLPs can be easily produced by simultaneously expressing the HA and NA along with a viral core protein, such as influenza M1 or retroviral Gag protein using a baculovirus-insect cell system (Haynes, 2009). The VLPs based on the M1 or Gag are highly immunogenic (Pushko *et al.*, 2005; Szecsi *et al.*, 2006) and can provide protection against virulent influenza viruses of the H1, H3, H5, H7 and H9 subtypes (Pushko *et al.*, 2005; Szecsi *et al.*, 2006; Matassov *et al.*, 2007; Quan *et al.*, 2007; Bright *et al.*, 2007, 2008; Mahmood *et al.*, 2008; Haynes *et al.*, 2009; Kang *et al.*, 2009; Perrone *et al.*, 2009; Ross *et al.*, 2009) and cross-protection from a heterovariant challenge in mouse and ferret models (Bright *et al.*, 2008; Mahmood *et al.*, 2008). The VLP vaccine based on baculovirus-insect cell system might offer advantages over traditional killed vaccines: improved immunogenicity and production systems without handling live virus. The above studies suggest that this technology could also be applied for SIVs.

### *Plasmid DNA-based vaccines*

DNA vaccines are naked DNA plasmids that have been genetically engineered to produce defined antigens within transfected cells. Intracellular antigens can be presented by Major histocompatibility complex (MHC) class I and II molecules, leading to stimulation of both humoral and cellular immune responses. DNA vaccines are an alternative to conventional killed vaccines and offer many advantages of the attenuated live vaccines without their potential risks (Olsen, 2000). The stable plasmid DNA can be easily produced on a large-scale at low costs. DNA vaccines have been tested for a wide variety of viral, bacterial and protozoal infectious pathogens (Kim and Jacob, 2009; Olsen, 2000). DNA vaccines for human and avian influenza viruses have been developed and good immune responses have been demonstrated in mice, chickens, ferrets, horses and non-human primates following the administration of HA, NP, NA and M constructs (Fynan *et al.*, 1993; Webster *et al.*, 1994; Liu *et al.*, 1997; Chen *et al.*, 2008, 1999a, b; Okuda *et al.*, 2001; Zhang *et al.*, 2005; Oveissi *et al.*, 2009; Yager *et al.*, 2009); clinical trials of DNA-based influenza virus vaccines are underway in humans (Drape *et al.*, 2006).

For swine influenza studies, two different DNA vaccine constructs have been used (Eriksson *et al.*, 1998; Macklin *et al.*, 1998). One study showed that the administration of the NP from the human influenza virus A/PR8/34 (H1N1) in pigs induced a strong humoral response but no detectable protection from virus challenge (Macklin *et al.*, 1998). In contrast, when pigs were administered a DNA vaccine with the HA gene from a H1N1 SIV (A/Swine/Indiana/1726/88), a decrease of virus shedding after challenge was observed (Macklin *et al.*, 1998). Larsen and Olsen (2002) showed that HA DNA vaccination induced strong priming of the humoral immune responses in pigs which can be significantly enhanced by increasing

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the vaccine dose. Co-administration of interleukin-6 DNA to pigs did not significantly improve immune responses to HA DNA vaccination or protection from challenge exposure (Larsen and Olsen, 2002). These results indicate that the administration of DNA plasmids encoding the HA gene from influenza viruses is an effective method for priming and/or inducing virus-specific immune responses, and for providing partial protection from a challenge infection in pigs (Macklin *et al*., 1998; Olsen, 2000; Larsen and Olsen, 2002).

Several safety concerns have been raised regarding the use of DNA vaccines. It was argued that DNA vaccines might integrate into host genomes, increasing the risk of malignancy and production of auto-antibodies against double stranded DNA leading to autoimmune disease (Kim and Jacob, 2009). However, to date, there has been no evidence of vaccine DNA integration into the host genome or the induction of anti-DNA antibodies. DNA vaccines are able to elicit broad-spectrum, long-lasting immunity through both humoral and cell-mediated immune reactions against influenza virus. DNA vaccines could be good candidates for swine vaccines, since they might provide heterosubtypic immunity and the internalization of DNA inside host cells would minimize interference by MDA (Thacker and Janke, 2008). However, a large amount of DNA is needed for vaccination and experimental trials of DNA vaccines in pigs have not been proven very successful. DNA vaccines might be useful as primer vaccines when followed by conventional inactivated vaccines (Larsen and Olsen, 2002). The need to develop more efficient delivery strategies that allow administration of DNA to easily accessible sites on the pig's body is a critical challenge for this technology and its clinical use in veterinary medicine.

## What is an ideal vaccine for swine influenza?

Each vaccine formulation has its own advantages and disadvantages. For example, killed vaccines are safe and provide good protection from genetically similar viruses, but lack heterovariant and heterosubtypic protection, might enhance disease and experience interference by MDA. Modified live-virus vaccines are able to provide good homosubtypic and partial heterosubtypic protection, do not enhance disease, but have the potential to reassort with circulating viruses. Antigenic shift and drift can cause vaccine failure in animals immunized with subunit vaccines. In most cases, vectored vaccines can only be applied once, i.e. the main target animals are only grow-finish pigs, not sows. Taken together, the choice of vaccines and immunization program is dependent on the epidemiological status in a swine herd and the age and future use of individual animals.

An ideal vaccine for swine influenza must overcome the difficulties encountered by traditional killed vaccines. Novel strategies have to keep up with the ever-evolving influenza viruses via updating virus seeds, overcoming interference from MDA, and providing broad homosubtypic and heterosubtypic protection. An ideal vaccine for swine influenza should be safe, easy to apply, cheap and able to prevent disease and virus shedding. In addition, one should be able to store the vaccine indefinitely at room temperature. An ideal vaccine should also have the following features: capacity of inducing effective herd immunity, one dose requirement, administration without a hypodermic syringe, and DIVA compatibility. To develop an ideal vaccine for swine influenza, future research is needed to address each of these areas. Currently, a priority for novel SIV vaccine development should focus on improvement of heterovariant and heterosubtypic immunity. There is only limited information on the extent of cross-protection between influenza virus variants or subtypes in humans and swine. In comparison to humans and mice, there is a big knowledge gap in swine immunology. Therefore, the cell-mediated and humoral immune responses at the systemic and mucosal levels need to be analyzed in future pig studies in order to develop better vaccines for the swine industry.

## Vaccine licensure

Since SIV is rapidly changing, continuous production and licensing of novel inactivated SIV vaccines is imperative. Because the efficacy of currently available killed influenza vaccines in swine is questionable, it is urgent to select novel vaccine seeds from currently circulating SIVs based on SIV surveillance data. It might be necessary to employ a similar vaccine strain selection system as used by WHO for human influenza vaccines to produce effective national and regional swine vaccines. However, there is currently no systemic surveillance for SIV in swine populations and no support by governments. In order to control swine influenza, procedures for new vaccine licensure need to be updated to keep pace with the fast changes in influenza virus genetics.

A swine influenza vaccine under U.S. Department of Agriculture (USDA) licensure procedures often takes up to 5 years to be licensed, which is much more laborious and expensive than for human influenza vaccines (Thacker and Janke, 2008). Therefore, national agencies [e.g. Center for Veterinary Biologics (CVB), part of USDA] need to streamline vaccine approval methods to enable timeliness of market entry for novel vaccines and updating of already existing vaccines. Recently, CVB changed its guidance on licensed killed swine influenza vaccines (Veterinary Services Memorandum No. 800.111), allowing up to two strain substitutions for each subtype at any one time without full-scale field safety tests. However, antigen concentration of each new strain must be not less than the strains in the licensed vaccine and manufacturing methods must be similar. Also, immunogenicity

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and efficacy must be demonstrated in an acceptable host challenge model.

Lessons from the current H1N1 virus pandemic teach us that influenza viruses are important zoonotic pathogens and surveillance for SIVs in pigs is necessary to prevent and control future pandemics. Therefore, national and international government agencies need to adjust policies on influenza surveillance and vaccine licensing in order to protect the public health and the swine industry.

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